AFRL-RX-TY-TM-2009-4551

ASSESSMENT OF THE PERFORMANCE OF IODINE-TREATED BIOCIDAL FILTERS AND CHARACTERIZATION OF VIRUS AEROSOLS

Jin-Hwa Lee

University of Florida
Gainesville, FL 32611

JULY 2009

Dissertation presented to the Graduate School of the University of Florida in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

AIRBASE TECHNOLOGIES DIVISION
MATERIALS AND MANUFACTURING DIRECTORATE
AIR FORCE RESEARCH LABORATORY
AIR FORCE MATERIEL COMMAND
139 BARNES DRIVE, SUITE 2
TYNDALL AIR FORCE BASE, FL 32403-5323

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

This report was cleared for public release by the Air Force Research Laboratory, Materials and Manufacturing Directorate, Airbase Technologies Division, Public Affairs and is available to the general public, including foreign nationals. Copies may be obtained from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

REPORT NUMBER AFRL-RX-TY-TM-2009-4551 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

JOSEPH D. WANDER, PhD
Work Unit Manager

DANIEL M. SAUCER, Major, USAF
Chief, Airbase Sciences Branch

ALBERT N. RHODES, PhD
Acting Chief, Airbase Technologies Division

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31-JUL-2009	Dissertation	30-JUN-2005 -- 17-JUL-2009

4. TITLE AND SUBTITLE

Assessment of the Performance of Iodine-Treated Biocidal Filters and Characterization of Virus Aerosols

5a. CONTRACT NUMBER

FA8650-06-C-5913

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

99999F

6. AUTHOR(S)

Lee, Jin-Hwa
University of Florida
Department of Environmental Engineering
P.O. Box 116450
Gainesville, FL 32611-6450

5d. PROJECT NUMBER

DODT

5e. TASK NUMBER

00

5f. WORK UNIT NUMBER

QL102008

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Florida
Gainesville, FL 32611

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/RXQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-RX-TY-TM-2009-4551

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution Statement A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

Ref AFRL/RXQ Public Affairs Case # 09-120. Document contains color images. Dissertation presented to the Graduate School of the University of Florida in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

14. ABSTRACT

Enhanced awareness of the threat of biological warfare and the spread of airborne pathogens has stimulated interest in bioaerosols and the need to develop better methods for respiratory protection. Among pathogens, viruses and bacterial spores are of special concern because they exhibit resistance to inactivation, small (highly penetrating) particle size and low median infectious dose. This study compared the mechanical and total viable removal by a relatively inefficient (~N50) iodine-treated biocidal filter challenged with aerosols of *Bacillus subtilis* spores and MS2 bacteriophage as surrogates for human pathogenic biological agents. The fate of viral aerosols influenced by environmental conditions and the spray medium were investigated by assessing infectious and total MS2 penetrating as a function of particle size, by comparing data from bioassay and polymerase chain reaction.

The iodine-treated electret filter has an viable removal efficiency for bacterial spores with a negligible pressure drop in various environmental conditions. Because of strong retention of bioparticles on the electret medium, inactivation of the collected spores is only slightly enhanced by the presence of the iodinated resin. In the viral aerosol experiment, the iodine-treated filter also showed high biocidal performance. Both and induced capture of iodine by viral aerosols traversing the filter and dissociation of free 1_2 are mechanisms consistent with the inactivation by I_2 observed under our experimental conditions, which included a 3-ppm background concentration of I_2 in the liquid impingers used for particle collection. Impinger studies using bovine serum albumin as a competitor for I_2 and of thiosulfate as an I_2 quench showed that the inactivation (Continued)

15. SUBJECT TERMS

pathogens, airborne, spores, aerosol, filtration, viral, infectious, influenza

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph Wander
U	U	U	UU	141	19b. TELEPHONE NUMBER *(Include area code)* 850-283-6240

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

SF 298, Block 14 (continued)

process is not immediate and that at least half of the iodine acting as a disinfectant was captured by bioaerosols as they penetrated the filter medium.

The distribution of infectious MS2 aerosols follows volume-based size distribution for relatively pure viral aerosols; meanwhile, solid-containing viral aerosols follow a dimension dependence of lower order, from 1st to 2nd power. Enumeration of infectious MS2 virions increases as relative humidity (RH) decreases and particle size increases owing to greater contribution of MS2 to the particle content. MS2 aerosols are relatively stable at low RH and become more susceptible at higher RHs, presumably due to the increased area of air/water interface. Aggregation results in a shielding effect and inert constituents yield an encasement effect because of reduced contact to the air/water interface. However, MS2 aerosols generated in artificial saliva appear to be attacked by the medium, and no protective effect of RH was observed.

ASSESSMENT OF THE PERFORMANCE OF IODINE-TREATED BIOCIDAL FILTERS AND CHARACTERIZATION OF VIRUS AEROSOLS

By

JIN-HWA LEE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

To my family in Korea, my son Luke, and my husband Youn-Sung Choi

ACKNOWLEDGMENTS

First and foremost I would like to gratefully and sincerely thank my advisor, Dr. Chang-Yu Wu, for his guidance throughout my graduate study with constant encouragement and insightful idea. This study would not have been completed without his patience and help.

I would like to convey my special thanks to my committee members. First, Dr. Joseph Wander gave me consistent advices and precious ideas to improve this study. Second, Dr. Dale A. Lundgren gave me valuable comments on my study and encouragement with generosity and kindness. Further acknowledgment is extended to Dr. Samuel Farrah, Dr. Jean M. Andino, Dr. Yiider Tseng, and Dr. Ben Koopman for their interest and suggestions on my research.

It was a great pleasure to work with fantastic colleagues, Alex D. Theodore, Qi Zhang, Danielle Hall, Brian Damit, and Seungo Kim, who work hard and have willingness to provide help at anytime. I would like to express my gratitude to Dr. Sewon Oh, a visiting professor from Sangmyung University, for his help and advice. My special thanks go to my friends, Myung-Heui Woo, Dr. Yu-Mei Hsu, Dr. Ying Li, Cheng-Chuan Wang, Anadi Misra, Charles Jenkins, and Lindsey Riemenschneider for their contribution to my study with interactive discussion, kindness that have made my study a delightful memory. I am also grateful to undergraduate students, Katie M. Wysocki, Christiana N. Lee, Ariana N. Tuchman, Diandra Anwar, Sang-Gyou Rho, and James Welch for their technical assistance and priceless help.

I dedicate this dissertation to my family. I would like to express my deepest and sincerest gratitude to my family in Korea for their endless love and support. Finally, my love and many thanks go to my greatest blessing, my husband, Youn-Sung Choi for his encouragement and consistent support, and my son, Luke Jun-Young Choi, who has grown up healthily.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ASSESSMENT OF THE PERFORMANCE OF IODINE-TREATED BIOCIDAL FILTERS AND CHARACTERIZATION OF VIRUS AEROSOL

By

Jin-Hwa Lee

August 2009

Chair: Chang-Yu Wu
Major: Environmental Engineering Sciences

The increasing threat of biological warfare and the spread of airborne pathogens have attracted the public's attention to bioaerosols and the need for the development of better methods for respiratory protection. Among biological agents, spores and viruses are of special concern because of resistance to inactivation treatment, small particle size and low infectious dose. In this study, the performance of an iodine-treated biocidal filter combining mechanical filtration and disinfection property of iodine was evaluated for *Bacillus subtilis* spores and MS2 bacteriophage as a surrogate for human pathogenic biological agents. Furthermore, the fate of viral aerosols influenced by environmental conditions and the spray medium were investigated by assessing infectious and non-infectious MS2 as a function of particle size with bioassay and polymerase chain reaction.

The iodine-treated filter has an excellent filtration efficiency for bacterial spores with a negligible pressure drop in various environmental conditions. Inactivation of the collected spores is only slightly enhanced by the presence of the iodinated resin. In the viral aerosol experiment, the iodine-treated filter also showed high biocidal performance. Both dissociation and capture iodine by viral aerosols traversing the filter are mechanisms responsible for the

inactivation. Significantly low pressure drop along with high viable removal efficiency imply its promising application as a respiratory protection device. The strong retention capability of the electrets filter minimizes reaeroslization but also makes it difficult to discriminate the antimicrobial effect at the surface.

The distribution of infectious MS2 aerosols follows volume-based size distribution for relatively pure viral aerosols; meanwhile, solid-containing viral aerosols follow a dimension dependence of lower size. Enumeration of infectious MS2 virions increases as relative humidity (RH) decreases and particle size increases owing to greater contribution of MS2 to the particle content. MS2 aerosols present stability at low RHs, while they are susceptible at higher RHs due possibly to the increased air/water interface. Aggregation results in shielding effect and inert constituents yield an encasement effect because of reduced contact to the air/water interface. However, for MS2 aerosols generated with artificial saliva, these protective effects cannot be distinguished.

CHAPTER 1
INTRODUCTION

Biological Threat

The perceived threat of bioterrorism after the anthrax attack on September 18, 2001 and airborne virus outbreaks, including historical epidemics of influenza, occurrences of SARS (severe acute respiratory syndrome), avian flu viruses, and more recently influenza A (H1N1), have drawn public attention to bioaerosols and protection methods. Biological agents have been used throughout the history as a weapon. In the 6th century B.C., Assyrians poisoned the wells of their enemies with rye ergot. In 1995, Aum Shinrikyo attempted on several occasions to release biological agents such as anthrax, botulinum toxin and ebola in aerosol form. Biological warfare agents can be made even by small groups and terrorist organizations because the production of bacteria, massive toxins and virulent strains of virus is easy and inexpensive. They can be more fatal threat than chemical weapons; a few kilograms of anthrax can kill as many people as a Hiroshima-size nuclear bomb (Prescott et al. 2002). Being invisible, odorless, and tasteless, biological agents can spread and remain undetected until symptoms are developed by infected people. Biological agents can be spread widely throughout a city or region, in contrast to chemical agents, which spread narrowly in downwind area near the point of release (Henderson 1999). *Bacillus anthracis,* one of the agents of concern listed by the Centers for Disease Control and Prevention (CDC), was used as a bioterrorism weapon in 2001 resulting in five deaths among the 11 people known to have inhaled it. Approximately 60 million dollars were spent to provide medical treatment to affected workers and to test and clean up the facility. It also resulted in the launching of "*Public Health Security and Bioterrorism Preparation and Response Act of 2002*" by the US government (MIPT 2002).

The spread of airborne pathogens is another emerging problem that increases the public's awareness of bioaerosols. For instance, SARS–a viral respiratory illness–is caused by a corona virus for which there is no vaccine. First reported in Asia in February 2003, SARS spread to more than two dozen countries in North America, South America, Europe and Asia over the following few months and resulted in deaths of 774 people amongst the total of 8,098 people infected worldwide. In addition, the more recent outbreak of influenza A (H1N1) virus sparked fears of a pandemic sweeping the world. Like SARS virus, there is currently no vaccine for the H1N1 virus and it is expected that people do not have immunity to this new virus (CDC 2009). Transmission of SARS and H1N1 viruses are suspected to occur through droplets generated from sneezing or coughing of an infected person, which subsequently deposit on or are transferred to the mucous membrane of the mouth, nose or eyes of nearby persons (CDC 2005). Besides these viruses, infection transmitted by the respiratory route include tuberculosis, mumps, measles, pneumonia, influenza, any many diseases not known to humans (Biswas and Wu 2005; Fiegel et al. 2006).

Bioaerosols

Even though interest in bioaerosols has recently been highlighted, bioaerosols have been present in the environment from the origin of mankind in both indoor and outdoor air. Bioaerosols are aerosols of biological origin – including viable bacteria, viruses, fungi and algae – as well as such nonviable materials as dust mites, pollen, endotoxins, mycotoxins and various allergens (Hinds 1999a). The size of bioaerosols ranges from aerodynamic diameters smaller than 0.5 μm to 100 μm (Cox 1995). Although the size of a single bacterium is commonly around 1 μm with various shapes such as spheres (cocci), rods (bacilli) or spirals, they are present in larger sizes as clusters or chains (Hinds 1999a). Larger bioaerosols are influenced by

gravitational force and are removed from air by settling in a short period of time. In contrast, smaller bioaerosols can remain in the air for a prolonged period of time and travel a considerable distances by themselves or attached to non-biological particles (*e.g.*, dust) in an air.

Various diseases such as tuberculosis, mumps, measles, rubella, pneumonia, meningitis, legionellosis, and influenza can be transmitted by bioaerosols (Jacoby et al. 1998). Bioaerosols need to be viable to be infectious, but viability is not a prerequisite to allergenic and toxic effects (Baron and Willeke 2001). Non-viable bioaerosols can also cause allergic reactions by contact and inhalation (Maus et al. 2001). Biological agents are also correlated with building-related illness (BRI) such as legionellosis and aspergillosis (Kemp et al. 1995b). Airborne transmission of respiratory diseases is classified into two groups: communicable and non-communicable. Communicable diseases can transmit between human hosts, while non-communicable diseases come only from the environment due to fungal or actinomycete spores and environmental or agricultural bacteria (Kowalski and Bahnfleth 1998).

Most terrestrial surfaces exposed to air movement can be potential sources of bioaerosols. Microorganisms in natural waters as well as anthropogenic water remain airborne after evaporation of the liquid resulting from rain, splashes, or bubbling processes. The growth and multiplying of microorganisms in a new environment of engineered systems such as humidifiers, evaporative air coolers, cooling coil drain pans, and condensation on ductwork insulation can result in an amplification of microorganisms to unhealthy levels (Kowalski and Bahnfleth 1998). Therefore, the heating, ventilation and air conditioning (HVAC) system of a building can be a major source of bioaerosols indoors (Baron and Willeke 2001). Workers in occupational environments where organic materials such as plants, hay, organic waste, wastewater, cotton and metalworking fluids are handled are exposed to high concentrations of bioaerosols. Through

sneezing and coughing, humans are also one of the most important sources of bioaerosols. Specifically, a single sneeze can generate a hundred thousand bioaerosols. A single cough produces only one percent of this amount, but 10 times more frequently than sneezes (Kowalski and Bahnfleth 1998). Thousands of droplets approximately 1 to 10 μm in diameter and containing viable microorganisms released by a person will quickly evaporate to droplet nuclei. For instance, the evaporation time of a 12-μm droplet is only 0.02 s. The droplet nuclei remain suspended in air for a long time and travel considerable distances by attaching to aerosols existing in air. Especially, respiratory viruses such as influenza virus appears to be spread mainly by droplet nuclei (Small 2002; Beggs 2003). Virus infectivity is shielded from drying, sunlight, and temperature compared to an isolated airborne virus due to encasement of droplet (Tyrrell 1967). In indoor environments, microorganisms are also free from factors inducing their destruction, thus resulting in longer survival. Direct sunlight has the potential to kill microorganisms since it contains a lethal level of ultraviolet radiation. Oxygen and pollutants in air may also be sources of the destruction of microbes. A study on the loss of viability of airborne microbes revealed that in the absence of sunlight, bacteria decay faster in air than viruses, because bacteria depend more on moisture for their survival than viruses do (Kowalski and Bahnfleth 1998).

Among the various biological agents, bacterial spores and viruses are of special concern because of their unique properties. In adverse environmental conditions, certain species of bacteria can survive by forming endospores exhibiting incredible longevity and resistance to environmental stress (Nicholson et al. 2000). Germination and the outgrowth of vegetative cells are initiated when the endospores encounter an appropriate environmental trigger, *e.g.*, a simple amino acid or riboside (Moir et al. 2002). Bacterial spores are highly resistant to deactivation,

such as by heat, radiation and chemical agents. Specific properties of spores are responsible for their resistance, including low water content in the core and saturation of the spore deoxyribonucleic acid (DNA) with a group of small, acid-soluble spore proteins (SASP) of the α/β-type (Popham et al. 1995; Tennen et al. 2000). Thus, bacterial spores have been classified as a group of bioagents for which treatment and disinfection are specially challenging.

Viruses are the smallest biological agents; a single naked virus ranges from 20 – 300 nm. However, in the natural environment, they are not typically present as a single naked virus due to aggregation of several single viruses or attachment to non-biological particles (*e.g.,* dust) in the air that result in several unique properties of their own (Hinds 1999a). Because of the surrounding outer layer of viruses, the inner viruses of an aggregate can be protected from inactivation treatments. This "shielding effect" of viral aggregates from inactivation treatment was already observed in water (Galasso and Sharp 1965). When viruses are attached or enclosed to the surrounding substances in the air, their infectivity can also be preserved by reduced contact with inactivation agents, *i.e.,* "an encasement effect". Second, the viral aggregates can be present in the ultrafine size range. Ultrafine particles are of great concern because respiratory deposition of a significant number of these particles is easily achieved. Behaviors in contrast to larger sized particles include translocation to extrapulmonary sites and migration to other target organs by a different transfer route (Oberdorster et al. 2005). Inhalation of aggregated ultrafine biological agents can result in fatal consequences, because only minute amounts of viruses are needed to cause disease (Hinds 1999a). For example, the infectious dose of smallpox is 10–100 viruses and that of viral hemorrhagic fever is 1–10 viruses (Pien et al. 2006). The low dose is estimated and true the infectious dose of many virus agents is unknown. Furthermore, a small viral particle can be suspended in air for a long time and travel considerable distances by itself or

by attachment to a non-biological particle resulting in a higher potential to spread disease. The other concern is that virions and even viral aggregates are in the size range of minimum filtration efficiency in air (usually 0.05 – 0.5 μm) (Hinds 1999b). Although the nominal MPPS (most penetrating particle size) designated by the NIOSH filter certification protocol is 0.3 μm, the reported MPPS of viral aerosols through N95 and N99 facepiece respirators was < 0.1 μm (Eninger et al. 2008b). Small particle size along with low infectious dose, high penetration through filter media, possible shielding effect of aggregation, and encasement effect of foreign substances are all challenges that anti-bioterrorism and public health workers need to overcome.

Stability of Viral Aerosols

In addition to the above properties, a key factor in determining the spread of disease by viral aerosols is their ability to survive and maintain infectivity, *i.e.*, the stability of viral aerosols (Cox 1995). The stability is influenced by compounds in the spray medium and environmental conditions such as temperature, relative humidity (RH), oxygen, and pollutant (Ehrlich et al. 1964; Songer 1967; Benbough 1971; Trouwborst and de Jong 1973; Schaffer et al. 1976; Ijaz et al. 1985; Hermann et al. 2007). Benbough (1969) investigated the effect of various compounds including NaCl, KCl, glucose, inositol, raffinose, glycerol, and bovine serum albumin on the survival of Semliki Forest virus (a group A arbovirus) and observed no affect of these compounds except NaCl. The removal of NaCl from the spray suspension led to better survival of viruses especially at high RH (HRH). In a following study (Benbough 1971), he observed that polyhydroxy-compounds protected arbovirus aerosols from virucidal effects of NaCl. The protective effect of polyols was also reported in another study of influenza A viral aerosols (Schaffer et al. 1976). Similar studies were conducted for human rotavirus by using tryptose phosphate broth and fecal matter; stability of viral aerosols at mid-range RH was observed (Ijaz

et al. 1985). In another study, the stability of coliphage T3 was reported when dextrose, spermine, spermidine−phosphate, thiourea, galacturonic acid, glucosaminic acid, and deuterium oxide were added to the spray medium (Ehrlich et al. 1964).

Among environmental conditions, RH is the most important when viral aerosols are generated by wet dissemination because dehydration is an inevitable condition (Cox 1989). Songer (1967) studied the effects of RH and temperature on various viral aerosols including Newcastle disease virus (NDV), infectious bovine rhinotracheitis virus (IBR), vesicular stomatitis virus (VSV), and *Escherichia coli* B T3 bacteriophage. All of the virus aerosols presented poorest survival at 35% RH; NDV and VSV survived best at 10% RH, while airborne IBR and T3 phage survived best at 90% RH. Individual variation of viral aerosols was also observed in another study where vaccinia, influenza and Venezuelan equine encephalitis (VEE) viruses were found to exhibit the best stability at 20% RH while poliovirus survived well at 80% RH (Harper 1961). Indeed, the effect of RH on infectivity of a wide range of viruses such as poliovirus, influenza virus, coliphage, porcine reproductive and respiratory syndrome virus (Harper 1961; Ehrlich et al. 1964; Songer 1967; Schaffer et al. 1976; Hermann et al. 2007) have been investigated. These authors concluded that lipid-enveloped viruses prefer low RH (LRH) but lipid-free viruses survive better at HRH. However, sensitivity to RH varied among virus aerosols depending on the individual characteristics of viruses. The molecular structure of virus is the key parameter that determines its stability and sensitivity to RH, and conditions under which the nucleic acid remains intact. For example, Dubovi (1971) successfully extracted infectious nucleic acid of MS2 and phi X 174 from inactivated viral aerosols, and Trouwborst and de Jong (1973) observed nucleic acid separated from protein coat during inactivation of viral aerosols at various RHs.

In these studies of stability of viral aerosols, the impingement device was extensively used as the sampling method (Ehrlich et al. 1964; Songer 1967; Benbough 1971; Trouwborst and de Jong 1973; Ijaz et al. 1985; Hermann et al. 2007). It collects the entire size range of generated viral aerosols. However, Hogan et al. (2005) observed that the collection efficiency of impingement devices depended on particle size and declined to less than 10% for lower submicrometer and ultrafine viral aerosols. As particle size increased, collection efficiency increased due to increased inertia. Therefore, collection by the impingement method is strongly biased toward the bigger particle size range of viral aerosols. This limitation of impingement methods, along with the fact that viral aerosols are present in the ultrafine and submicrometer range, may lead to inaccurate understanding of the state of the viruses. How viruses are distributed in aerosol particles as a function of particle size is a critical piece of information. In this context, Hogan et al. (2005) explored the distribution of viruses for three particle sizes, 25 nm, 150 nm, and 300 nm. However, critical parameters that affect the infectivity of viral aerosols, such as environmental conditions and composition of the fluid medium in which they are suspended, were not considered.

Polymerase Chain Reaction Analysis

As one of analytical tools for bioaerosols, polymerase chain reaction (PCR) is a method to detect microorganisms even from small quantities of sample by amplifying a target nucleic acid sequence of DNA, which confers advantages of sensitivity and rapidity over traditional culture methods (Alvarez et al. 1995). Since PCR relies on DNA information, the PCR value is the quantity of total microbial DNA – including viable and nonviable microbes without concern about viability. For microbes having only ribonucleic acid (RNA) not DNA, Reverse Transcription-PCR (RT-PCR) is widely used to detect and amplify RNA by producing DNA

complementary to the RNA, called copy DNA (cDNA) (Mackay 2004). The PCR protocol has three steps which depend on the temperature cycling: (1) the double stands of DNA are melted at 94-96 °C yielding two single stands of DNA (denaturation); (2) the primers anneal to the single–stranded DNA by making hydrogen bonds at 50-60 °C (annealing); and (3) DNA polymerase synthesizes new DNA strands complementary to the DNA template strands by incorporating the deoxyribonucleotide triphosphates (dNTPs) at 70–74 °C (elongation). At this increased temperature, the mismatched bases of DNA will be detached due to not having hydrogen bonds enough to withstand the increased temperature. Repeated cycles of denaturation, annealing, and elongation quickly amplify the sequence of interest exponentially (Lodish et al. 2003). The amount of amplified product by this protocol is observed by fluorescence signal, caused by incorporating a probe. The probe containing both a reporter dye and a quencher is complementary to the target sequence. During the elongation step, polymerase cleaves the probe, releasing the reporter away from the quencher. Therefore, the fluorescence intensity of the reporter dye is proportional to the amount of amplification (O'Connell et al. 2006).

Filtration

Filtration is the most common method for aerosol removal and has been used extensively in HVAC systems as well as in respiratory protection devices with the advantages of simplicity, versatility and economical collection of aerosols (Hinds 1999b). The ability of filters to collect particles is described by their *collection efficiency,* defined as the fraction of impinging particles retained in the filter, and *pressure drop,* which is related to energy cost. The five basic mechanisms associated with filtration collection are inertial impaction, diffusion, interception, gravitational settling, and electrostatic attraction. Large particles unable to quickly adjust

themselves to the changing gas streamline near the fiber will cross the streamline and hit the fiber by inertial impaction. Depending on the ratio of terminal settling velocity of particle and face flow velocity, very large particles are deposited on the filter by gravitation settling. In contrast, small particles encounter the fiber due to Brownian motion. When particles follow the streamline perfectly (*i.e.*, they have negligible inertia, gravitational settling and Brownian motion), they are collected by interception on the filter fiber due to its finite size. Lastly, charged particles, charged fibers, or both induce electrostatic attractions (*i.e.*, coulombic forces and image forces) and result in particle deposition on the filter (Hinds 1999b).

Aerodynamic diameter (equivalent diameter to a spherical particle with a unit density of $1g/cm^3$) is the key parameter in characterization of filtration (Hinds 1999d); nevertheless, it is not the only factor to be considered in the collection of bioaerosols. The physical properties of microorganisms—including shapes of aerosols and surface structure—are also important factors in collection on the filters. According to Qian et al. (1998), penetration by polystyrene latex spheres was higher than that of *Micobacterium chelonae*, a rod-shaped bacterium, of similar aerodynamic size. A similar study reported that the penetration by rod-shaped organisms was lower than that by spherical organisms (Willeke et al. 1996). In another study (Jankowska et al. 2000), slightly lower collection efficiency of various filter media for fungal spores than that of potassium chloride (KCl) particles of the same aerodynamic size was reported due to breakup of spore aggregates, which is different among various fungal spore species depending on the surface such as spiny structure.

When fiber materials are used in HVAC system as well as in respiratory protection, they collect various substances in the air including dust as well as bioaerosols. The collected bioaerosols can remain viable or proliferate under suitable growth conditions such as sufficient

nutrient, proper humidity and temperature (Maus et al. 2001). The colonization of bacteria and fungi in the air filter used in the HVAC system was observed in previous studies (Kemp et al. 1995a; Kemp et al. 1995b; Simmons and Crow 1995). Furthermore, the survival of bacteria in various types of surgical masks and respirator filters was also documented (Brosseau L. M. et al. 1997). It has been also shown that building materials such as wallpaper and gypsum boards can be a source of microbial air contamination when the growth of microorganisms is supported by sufficient moisture and nutrients (Nielsen et al. 1998). Studies on the growth and survival of microorganisms on two different air filtration media reported the survival of a wide range of fungal species and bacteria on the fiberglass medium that had significantly high water content. After 6 weeks of use, the accumulation of dust on the multi-layered polymer material provided nutrients for the growth of microorganisms (Foarde et al. 1996). However, the growth of microorganisms can be inhibited when the growth medium dries out, suggesting better survival of microorganisms at a favorable RH (Heldal et al. 1996). Other researchers observed that microorganisms did not multiply in unused filter media at low RH (RH < 70%) but the growth of microorganisms was induced only where sufficient moisture and nutrients were possessed (Kemp et al. 1995a). Simmons and Crow (1995) observed fungal growth at HRH (> 70–80 %), which was supported by the presence of air dust and/or cellulose fibers in the filter. Regarding respirators, rapid fungal growth in the respirator made of cellulose was observed in the humid storage environment (Pasanen et al. 1993). When the respirator is worn for several hours, the humidity and nutrients in the respirator may be increased due to exhalation and saliva containing various components, which can be either nutrient or antimicrobial agents (Wang et al. 1999).

Microorganisms collected on filter are of great concern due to microbial contamination of ambient air by releasing byproducts or by their re-entrainment along with the adverse health

effects of bioaerosols. Various studies (Qian et al. 1997; Willeke and Qian 1998; Wang et al. 1999) reported re-entrainment of the surviving microorganisms on the filter into the air passing through the filter media. Even though an HVAC system prevents the contamination of indoor air by microbial contaminants entering from outdoors, once their growth occurs in the system, they appear in returned air at a higher level than in the outdoor air (Kowalski and Bahnfleth 1998). Similar studies also demonstrated that air conditioning systems contribute to increased microbial concentrations in ventilated rooms (Hugenholtz and Fuerst 1992). For respirators, a study (Qian et al. 1997) on the re-entrainment of bacteria and solid particles from N95 respirators observed that re-entrainment of particles smaller than 1 μm does not exceed 0.025 % at low RH, even at high air velocity corresponding to violent sneezing and coughing (*e.g.*, 300 cm/s). Meanwhile, re-entrainment of larger particles into air is significant at the same re-entrainment velocities and low RH level of 22 %. The larger particles can be aggregates of bacteria or the attachment of bacteria to large inert particles. The other study (Jankowska et al. 2000) compared the re-entrainment of biological particles to that of inert particles from the filter and reported that the re-entrainment of fungal spores was higher than that of KCl particles due to deaggregation of fungal spores. Due to re-entrainment and resistance caused by microbial growth, the performance of a filter can deteriorate over time. Therefore, special care should be taken in handing, storage, or reuse of the filters, and frequent inspection and maintenance should be conducted. From these two perspectives, one being the prevention of contamination of ambient air by re-entrained microorganisms and the other being the extension of the lifetime of the filtration system by preventing proliferation of microorganisms in the filter, it is imperative that collected microorganisms are deactivated. Therefore, in recent years, there have been efforts to

incorporate antimicrobial materials into air filters to destroy or inhibit the growth of microorganisms (Foarde et al. 2000; Cecchini et al. 2004).

Iodine as a Disinfectant

Elemental halogens (Cl_2, Br_2, I_2, etc.) exist as diatomic molecules and form saltlike compounds with sodium and other metals (Prescott et al. 2002). Chlorine and iodine have the characteristics of antimicrobial agents. Chlorine is the most commonly used disinfectant in water treatment among diatomic halogens due to its relatively low cost. However, unacceptable residual levels of chlorine are a possible disadvantage of using chlorine as a water disinfectant. Iodine is used by military, in developing countries, and in emergency or temporary use for portable water purification. It is superior to chlorine due to the greater chemical stability of the product and less reactivity with organic nitrogenous contaminants in water (Bruchertseifer et al. 2003). Moreover, iodine is very stable in water over a wide *p*H range (6–8) and has low solubility in water. However, continuous consumption of iodine-treated water is not recommended due to its adverse health effect.

In aqueous solution, iodine may exist as various species (*e.g.*, I^-, I_2, I_3^-, I_5^-, I_6^{2-}, HOI, OI^-, HI_2O^-, I_2O^{2-}, H_2OI^+, and IO_3^-) since iodine can form compounds in all oxidation states from –1 to +7 (Gottardi 2001). The overall reaction of iodine in water starts from hydrolysis to form hypoiodous acid (HOI) as shown in Eq. (1-1). Hypoiodous acid then disproportionates to iodate (IO_3^-) as depicted in Eq. (1-2). Equation (1-3) presents the overall reaction by combining these two reactions. According to this equation, iodine molecules are significant in acidic conditions. In neutral and basic solution, iodide and triiodide co-exist as shown in Eq. (1-4). At high *p*H (>10), HOI dissociates to hypoiodite ion (OI^-) and hydrogen ion (H^+) as shown in Eq. (1-5) (Bruchertseifer et al. 2003).

$$I_2 + H_2O \leftrightarrows I^- + HOI + H^+ \quad (1\text{-}1)$$

$$3HOI \leftrightarrows 2I^- + IO_3^- + 3H^+ \quad (1\text{-}2)$$

$$3I_2 + 3H_2O \leftrightarrows 5I^- + IO_3^- + 6H^+ \quad (1\text{-}3)$$

$$I_2 + I^- \leftrightarrows I_3^- \quad (1\text{-}4)$$

$$HOI \leftrightarrows H^+ + OI^- \quad (1\text{-}5)$$

Among the various iodine species, iodine molecules and hypoiodous acid have disinfection capability (Chang 1958). While hypoiodous acid is the most effective form of disinfectant, molecular iodine is important in the inactivation of microorganisms due to its stability over a wide *p*H range compared to hypoiodous acid (Brion and Silverstein 1999). It is speculated that iodine molecules penetrate the cell wall of microorganisms and inflict structural damage on the capsid protein (Maillard 2001). Oxidation of sulfhydryl (-SH) groups or substitution onto tyrosine and histidine residues results in the disruption of normal functions of these amino acids (Carroll 1955). Brion and Silverstein (1999) observed changes in the isoelectric focusing points of MS2 virions after iodine treatment from acidic *p*H value to more basic values, verifying that conformational changes occur in the protein of MS2 bacteriophage. The bactericidal and virucidal properties of iodine were observed by Hsu et al. (1965). On the other hand, iodine does not inactivate either infectious ribonucleic acid (RNA) or DNA (Hsu 1964). Meanwhile, study on the sporicidal effect of iodine on *Bacillus metiens* spores showed a decrease of germicidal activity due to increased iodine decomposition (Wyss and Strandskov 1945). Generally, iodine inactivation is effective in clean water, at higher *p*H, at higher temperature and at higher iodine dose. When using iodine as the disinfectant for such fluids as water and air, care should be exercised due to the risk of iodine vapor ingestion and concern for

hypothyroidism. Iodine vapor is intensely irritating to mucous membranes and adversely affects the upper and lower respiratory system (ACGIH 2001). The inhalation of iodine causes coughing, burning sensations to the mucosal, tracheal, and pulmonary tissues, and tightness in the chest because it increases airflow resistance in the lungs by reducing the ability of the lungs to take up oxygen. Intense exposure to iodine may lead to lung disease and affect the central nervous system (OEHHA 2003; ATSDR 2004). Below the threshold limit value (*i.e.*, 0.1 ppm) of iodine, humans can work undisturbed. However, discomfort can be encountered at 0.15–0.20 ppm and work is impossible at concentration of 0.30 ppm (Cameron 2002).

Iodinated Resin Filter Media

Iodinated resins have been developed to provide demand-on-release of iodine residuals for disinfection. Iodine can be attached to a quaternary ammonium strong base anion exchange resin in the form of triiodide (I_3^-) and pentaiodide (I_5^-) ions (Marchin et al. 1997). The performance of the triiodide and pentaiodide resin was evaluated for microorganisms and 4-log inactivation of bacteria and viruses were reported (Fina et al. 1982; Marchin and Fina 1989). Marchin et al. (1983) reported greater disinfection efficacy of pentaiodide resin for cysts than that of triiodide. Later, the authors also observed better performance of pentaiodide for disinfection of *Escherichia coli* in both normal and microgravity (Marchin et al. 1997). Although the iodine resin typically produces a residual of 0.02 – 2.00 mg I_2/L in water passing through the filter, significantly higher iodine residual concentration (*i.e.*, 9 times) in the effluent of the pentaiodide resin than that of the triiodide resin was reported suggesting the need of a carbon filter to capture the residual iodine (Fina et al. 1982; Marchin and Fina 1989). These studies indicate that the presence of pentaiodide ions on the resin will lead not only to greater disinfection efficiency but also to an increased level of iodine vapor downstream of the iodinated

resin compared to the triiodide resin. The iodinated resin filter, as an electret filter, is expected to possess high removal efficiency and lower pressure drop than conventional filter media. Negatively charged microorganisms attracts polarizable iodine complexes on the filter during near-contact encounters to transfer iodine molecules (Ratnesar-Shumate et al. 2008). Studies on the disinfection capacity of iodinated resin filters for the treatment of bacteria and viruses in water were conducted three decades ago and reported disinfection capacities over 99.99% (Taylor et al. 1970; Gilmour and Wicksell 1972; Hatch et al. 1980; Fina et al. 1982; Marchin et al. 1997). However, only limited studies have been conducted on the disinfection capacity of iodinated resin filters for air treatment recently (Messier 2004; Heimbuch and Wander 2006; Heimbuch et al. 2007; Eninger et al. 2008a; Lee et al. 2008; Ratnesar-Shumate et al. 2008; Messier 2009).

In the previous study (Ratnesar-Shumate et al. 2008), the high removal efficiency of iodine-treated filter was demonstrated for vegetative cells including *Escherichia coli* and *Micrococcus luteus*. The authors also proposed a near-contact transfer mechanism between the iodine-treated filter and microorganisms penetrating the filter as an inactivation mechanism, but without solid proof. To increase the reliability of the iodine-treated filter as a protective device against airborne pathogens and biological agents, studies on more resistant microorganisms and microorganisms of the smallest size are needed. Furthermore, investigation of the viability of microorganisms collected on the filter is a critical step to prove the disinfection capacity of the iodine-treated filter. Both relative humidity and temperature are important environmental factors that influence the performance of iodine-treated filters because it is expected that the disinfection efficacy of an iodine-treated filter will be increased due to the dissociation and dissolution of iodine at higher temperature and RH. These factors and conditions need to be taken into account

in the evaluation of an iodine-treated biocidal filter to assess its potential use as a reliable respiratory protective device. Furthermore, a second possible source of inactivation mechanisms—I_2 released from the filter—along with the proposed near-contact transfer mechanism needs to be considered. I_2 released from the filter can cause inactivation in the sampling device, whereas I_2 captured by microorganisms passing through the filter can inactivate them in their airborne state and/or continue the inactivation process after collection in the sampling device, either bound to the particle or by dissolving into the aqueous medium. Identification of inactivation by dissolved I_2 could confound the results in earlier reports (Messier 2004; Heimbuch and Wander 2006; Heimbuch et al. 2007; Eninger et al. 2008a; Lee et al. 2008; Ratnesar-Shumate et al. 2008) that used plating methods to measure viable removal efficiency, which would require an independent experimental method to quantify the relative importance of two competing inactivation mechanisms.

Research Objectives

Two objectives were pursued in this doctoral study to address the challenges mentioned above:

(1) Performance of iodine-treated biocidal filter media as a protective gear against biological agents and airborne pathogens under various environmental conditions were evaluated. To achieve this objective, a filtration system was used to investigate the removal efficiency of filter media and vortexing experiment was conducted to assess the viability or infectivity of biological agents collected on the filter. Furthermore, an inactivation mechanism of the iodine-treated biocidal filter was assessed.

(2) MS2 bacteriophage aerosols in the ultrafine and submicrometer range were characterized by investigating infectious and non-infectious virions as a function of particle size to

understand the distribution of viruses in the airborne state. Furthermore, the effect of relative humidity and spray medium on the infectivity of viral aerosols was explored.

Specifically, four tasks were carried out:

(1) evaluate physical and viable removal efficiency of the iodine-treated filter for bacterial spores and viral aerosols

(2) investigate viability and infectivity of biological agents collected on the iodine-treated biocidal filter.

(3) assess the inactivation mechanism of the iodine-treated biocidal filter by using various reaction solutions.

(4) characterize size-classified viral aerosols influenced by relative humidity and the spray medium by using plaque assay method and PCR analysis.

CHAPTER 2
EFFICACY OF IODINE-TREATED BIOCIDAL FILTER MEDIA AGAINST BACTERIAL SPORE AEROSOLS

Objective

The objective of the study presented in this chapter was to evaluate the performance of an iodine-treated biocidal filter for bacterial spores in various environmental conditions. Viable removal efficiency (VRE), pressure drop (ΔP), and the viability of collected microorganisms on the iodine-treated filter were investigated and compared with those of the untreated filter.

Materials and Methods

Filter Media

The iodine-treated filter (JT-70-20XP-10T-100) and untreated (JT-70-20XP-100) media tested in this study as discs of 47–mm diameter and 2 mm in thickness were provided by Triosyn® Corp. Triiodide, prepared from stoichiometric amounts of I_2 and potassium iodide mixed with a minimum amount of water, was contacted with a quaternary ammonium anion exchange resin to substitute the anion with triiodide. Due to the charges on the fibers, these filters are classified as electret filters. Details of the preparation are available in the patent by Messier (2004). The iodine concentration in effluent air passing through the iodine-treated filter can be measured by the OSHA analytical method (ID-212) for iodine in workplace atmospheres. The iodine sampled in the impinger medium (1.5 m*M* Na_2CO_3 and 1.5 m*M* $NaHCO_3$) can be analyzed as iodide by ion chromatography (OSHA 1994). The measured iodine concentration was 0.004 mg I_2/m^3.

Test Microorganisms

Bacillus subtilis vegetative cells were supplied by the Department of Microbiology and Cell Sciences at the University of Florida for the production of *B. subtilis* spores. *B. subtilis* is a Gram-positive, non-pathogenic, rod-shaped bacterium 2.0–3.0 μm in length and 0.7–0.8 μm in

width (Prescott et al. 2002). *B. subtilis* spores are commonly used as a surrogate for *B. anthracis* spores, which were the bioterrorism agent used in 2001 (CDC 2004). For sporulation, the African violet method (African violet soil 77.0 g, Na_2CO_3 0.2 g, and sterile deionized (DI) water 200.0 mL) suggested by the American Type Culture Collection was used (ATCC 1998). The agar was prepared by mixing nutrient agar with 25% extract of African violet soil and 75% sterile DI water. *B. subtilis* was inoculated in this agar slant and incubated at 36 ^{o}C for one week to produce spores, which are 0.8 ~ 1.2 μm in length with either a spherical or ellipsoidal shape (Ricca and Cutting 2003). After spore production, bacterial growth was harvested into 2 mL of sterile DI water and poured into a sterile glass tube. The glass tube containing the spore suspension was heated in a water bath at 80 ^{o}C for 30 mins to kill vegetative cells. After cooling, the spore suspension was diluted with 5 mL of sterile DI water and centrifuged at 3500 rpm (Model 225, Thermo Fisher Scientific Inc., Atlanta, GA, USA) for 5 mins. The supernatant consisting of cell debris was then removed. This process was repeated twice more, and the spores were resuspended in 5 mL of sterile DI water. After this purification process, the spore suspension was stored in a refrigerator at 4 ^{o}C before experimentation. Microscopic observation of the spore suspension after applying the malachite green spore-staining technique (Munro 2000) demonstrated the purity of the culture by showing the majority to be endospores, with only a minute amount of cell debris.

Experimental System

The experimental system for evaluating the removal efficiency is shown in Figure 2-1. A six-jet Collison nebulizer (Model CN25, BGI Inc., Waltham, MA, USA) was used to aerosolize the spore suspension with a flow rate of 7 Lpm (liters per minute). The spore suspension in the nebulizer was made by dispersing 0.1 mL of purified spore suspension in 150 mL sterile DI

water. The aerosolized suspension was dried with filtered compressed air in a 2.3-L glass dilution chamber. A flow rate of 15 Lpm, which corresponds to a face velocity of 14.2 cm/s, was used and controlled by a calibrated rotameter. Based on the velocity, flight time through the 2–mm filter is estimated to be 14 ms. This face velocity, used by Triosyn Corp., corresponds to certification testing of 100 cm^2 media (Di Ionno and Messier, 2004) at the 85–Lpm flow rate suggested by the National Institute for Occupational Safety and Health (NIOSH 2005). The concentration of bacterial spore aerosol for challenging the test filter was 1.2×10^4– 3.2×10^4 colony-forming units (CFU)/m^3. Pressure drop across the test filter disc was monitored using a Magnehelic gauge measuring 0–10 in. H_2O and recorded every 20 minutes. An Andersen six-stage viable impactor (Model 10-820, Thermo Electron Corp., Waltham, MA, USA) was used to classify generated bacterial spores and those that penetrated the test filter. After sampling, glass Petri dishes filled with nutrient agar were removed from the impactor, reversed, and incubated for 24–36 hrs for enumeration of microorganism growth. A glass fiber filter (AP 1504700, Millipore Corp., Bedford, MA, USA) was placed downstream of the impactor to capture spores not collected by the sampler, if any, to prevent contamination of ambient air. Because the cut size of the sixth stage of the impactor (0.65 μm) is smaller than the nominal size of a *B. subtilis* spore (1 μm), it is unlikely that any spores remained in the downstream air of the impactor. However, spore fragments that were not removed by the impactor were removed by the downstream filter. The experiments were conducted at three environmental conditions: room temperature (23±2 ^oC) and low RH (35±5 %) (RT/LRH), room temperature and high RH (95±5 %) (RT/HRH), and high temperature (40±2 ^oC) and HRH (HT/HRH). An increased disinfection efficacy of iodine was expected at high temperature and high RH due in part to iodine's sublimation and dissolution. For the experiments at high temperature the dilution dryer was

wrapped in an electronically controlled heating jacket. High RH was achieved by adding humid dilution air to the system.

Viable Removal Efficiency

The VRE of the test filter was calculated by enumerating bacterial growth in agar plates of two impactors, one downstream of the test filter and the other for control, which has no test filter. The VRE was determined by using Eq. (2-1).

$$\text{VRE (\%)} = \left(1 - \frac{N_p}{N_t}\right) \times 100 \tag{2-1}$$

where N_t is the total number of viable spores collected in the control and N_p is the number of viable spores collected downstream of the test filter. The entering bioaerosol concentration was measured by collecting spores at all six stages of the impactor with no test filter for the first and last 5 mins of an experimental run. The run time of 5 mins was chosen to prevent overloading of spores on the agar. The average number CFU of the two measurements was used to determine the entering bioaerosol concentration for 2 hrs of experimental run. Due to the expected low penetration of spores through the test filter, the impactor downstream of the test filter contained only the sixth-stage agar plate. The agar plate was replaced with a fresh one every 20 mins for 2–hrs to avoid overloading and dehydration of the agar. Five 2-hr trials were conducted—the total evaluation time for each filter was 10 hrs—and three filters were tested (*i.e.*, 15 trials). However, due to the stability of results seen at RT/LRH and time constraints, only two iodine-treated filters were tested for two 2-hr runs in other environmental conditions (*i.e.*, four trials). Agar plates containing more than 300 colonies were counted following the positive hole method recommended by the manufacturer (Thermo Electron Corporation 2003).

Viability of microorganisms on the filter

After the filtration experiment, the test filter disc was removed from the filter holder in the experimental apparatus and subjected to the vortexing experiment to determine the viability of the spores collected on the filter. The filter was immersed in 40 mL of sterile DI water in a 250–mL beaker and agitated with a vortex mixer (Model M16715, Barnstead, Dubuque, IA). After 1 min of vortexing, 1 mL of sample was withdrawn for measuring the viability of the extracted spores in the original solution, and another 1 mL was withdrawn and measured after appropriate dilution (10^{-n}). The same procedure was repeated after 2, 3, 5, and 10 mins of vortexing time without changing the solution. Thus, the count of extracted spores, C_E was determined by using Eq. (2-2).

$$C_E = \frac{CFU}{10^{-n}} \times \frac{V_1}{V_2} \tag{2-2}$$

where CFU is the number of colony-forming units counted, V_1 is the volume of extraction fluid (1 mL), V_2 is the volume of diluted suspension spread on the agar plate (1 mL), and n is the dilution factor. The total viability of the extracted spores was calculated by averaging the number of viable spores at all vortexing times. To compare the results of the iodine-treated and untreated filters, we defined survival fraction as the ratio of the extracted spores in the vortexing solution to the spores collected on the test filter (C_E/C_C). C_C is determined by the total count in the control impactor multiplied by the VRE of the filter. In aqueous solution, the resin surfaces may release iodine molecules that also may deactivate spores. This reaction raises concerns that spores can be deactivated in the vortexing solution by free residual iodine rather than deactivation solely on the filter. To investigate this possibility, the solution after vortexing a clean iodine-treated filter at each designated time was inoculated with a spore suspension of known concentration. After 10 mins of exposure time, spore concentration was measured to determine the free residual iodine effects. The concentration of iodine in the vortexing solution

was also examined by the DPD (*N*, *N*-diethyl-*p*-phenylenediamine) colorimetric method adapted from *Standard Methods for the Examination of Water and Wastewater 4500-CI* (APHA 1995). Ten mL of solution vortexed with the iodine-treated filter was analyzed at 530 nm by using a DR/4000 V Spectrophotometer (Hach, Loveland, CO, USA). Iodine in the solution reacts with DPD forming a pink color, the intensity of which is proportional to the total iodine concentration (Hach 2003). The effect of vortexing alone on the viability of spores was also investigated by following the same vortexing procedure with a spore suspension of known concentration.

Results

Removal Efficiency

Figure 2-2 shows the size distribution of the entering spores collected by the impactor. As shown, the majority of the entering spores were in the 0.65 ~ 2.1-μm range, indicating they were predominantly singlets. As shown in Table 2-1, both iodine-treated and untreated filters displayed a high VRE (> 99.996 %) at RT/LRH due to the high mechanical removal efficiency. Differences in the VRE should be distinguishable at a much higher upstream concentration, but this would overload the impactor and filter in our experimental configuration. In other environmental conditions (*i.e.*, RT/HRH and HT/HRH), the iodine-treated filter also achieved high VRE (> 99.998 %). It should be noted that even when the filter did not show complete removal, in most cases only one or two CFU penetration was detected downstream. There was no difference in any 2-hr interval, indicating that the performance did not deteriorate over time during the 10-hr or 4-hr experimental runs. Raw data are presented in Appendix A.

Pressure Drop

Since pressure drop is an important parameter in practical applications, ΔP was recorded every 20 minutes. Under the operating condition, the initial pressure drop was approximately

423 Pa (at 14.2 cm/s) and was maintained throughout the entire experiment with almost negligible variation. There was no observable difference in pressure drop between the iodine-treated and untreated filters.

Survival Fraction

To determine the viability of the collected spores, both iodine-treated and untreated filters were vortexed to extract spores from the filters. A larger number of spores extracted from the untreated filter was enumerated than from the iodine-treated filter at RT/LRH. No increase of extracted spores from the test filters was observed as the vortexing time increased. Although both survival fractions were low, the survival fraction of the iodine-treated filter was significantly lower than that of the untreated filter, which was confirmed by *t*-test (*p*-value < 0.05). At RT/HRH and HT/HRH, the survival fraction of the iodine-treated filter showed around one log unit higher value than that at RT/LRH. This higher survival fraction can possibly be explained by the loss of iodine from the filter due to increased sublimation of iodine at HT and dissolution through the hydrolysis of iodine at HRH. To test this hypothesis, we measured the iodine concentration in the vortexing solution of the iodine-treated filter by the DPD colorimetric method. The values (mg I_2/L) at RT/HRH (0.40 ± 0.03) and HT/HRH (0.30 ± 0.03) were lower than that at RT/LRH (0.90 ± 0.03). Statistical significance between RT/LRH and the others was observed by performing one-way ANOVA (*p*-value < 0.05). Meanwhile, the difference between RT/HRH and HT/HRH was not significant (*p*-value > 0.05). We note that *n* is small (*i.e.*, 2) and measurement uncertainty of survival fractions is large at both RT/HRH and HT/HRH.

To investigate the effect of residual free iodine in the vortexing solution on the survival fraction of the extracted spores, spores were inoculated into the solution after vortexing a clean

iodine-treated filter at each designated vortexing time. As shown in Figure 2-3, the effect of the extracted iodine did not increase as vortexing time increased. The average (±S.D) fraction of spores was 0.856 (±0.014) and 1.01 (±0.03) in the iodine-treated and untreated solution, respectively, indicating that the iodine extracted from the iodine-treated filter during vortexing decreased the viability of spores in the solution by ~15%. Accordingly, the survival fraction of spores on the iodine-treated filter was corrected by this amount.

The effect of vortexing alone on the viability of spores was also examined. A spore suspension of known concentration was vortexed for each designated time, after which the viability of each was examined. The relative fraction obtained by dividing the number of viable spores after each vortexing time with that at zero vortexing time was calculated. The average (±S.D) fraction was 1.03 (±0.15), demonstrating that 10 mins of vortexing had a negligible effect on the viability of spores. The corrected survival fraction considering only the effect of free residual iodine is presented in Table 2-2.

After vortexing, a tested and an unused iodine-treated filter were examined under a scanning electron microscope (SEM) (FESEM-6335F, JEOL, Japan) to look for spores not extracted from the filter. As shown in Figure 2-4, a few micron-sized particles remained in the tested iodine-treated filter, whereas the unused filter was free of particles.

Discussion

In practical applications, a desirable filter medium will provide high aerosol removal efficiency with an acceptable ΔP depending on the applications. For a ventilation system, a large ΔP imposes high energy and maintenance costs. In respiratory protection, a large ΔP translates into breathing exertion with a respirator, which may impair the agility or compromise the mobility and endurance of personnel in the battlefield or workers in disaster zones. The test

filter medium can be applicable to both ventilation systems and respiratory protection devices. Since the pressure drop of the filter is directly proportional to face velocity with the assumption of laminar flow inside the filter (Hinds 1999b), the expected pressure drop of the test filter at a face velocity of 5.3 cm/s is around 157 Pa, which is much less than the military standard of 392 Pa for HEPA filter media (U.S. Army 1998). In the case of respirator application, the pressure drop of the test filters can be calculated for the face velocity of 7.8 cm/s, which is achieved when the flow rate of 85 Lpm for the NIOSH respirator certification testing is applied to commercially available facepiece respirators (Barrett and Rousseau 1998). The calculated pressure drop of the test filters is 224 Pa, which is much less than the inhalation resistance of 343 Pa permitted by NIOSH for certified respirators (NIOSH 1995). Incorporation of iodine on the resin filter media did not affect ΔP. The test filter media exhibited high VRE (> 99.996%) for bacterial spores. This value is as high as the filtration efficiency of NIOSH-approved N95 and P100 respirator filters for *B.globigii* spores (99.87 % and 99.98 %, respectively) using 85–Lpm flow rate as reported in a recent study (Richardson et al. 2006).

There are great concerns about the growth of microorganisms collected on the filter, which may result in the release of byproducts and re-entrainment. It also poses a hazard to workers who handle the disposal of a microorganism-loaded filter. It has been shown that fibrous building materials—including insulation substances and ceiling tile—serve as nutrients for the growth of microorganisms under sufficient relative humidity (Ezeonu et al. 1994; Chang et al. 1995). Research about the effect of air filter media on the viability of bacteria showed that fiber materials did not have an inhibitory effect on the survival of microorganisms even if they do not grow (Maus et al. 1997). Sensitive cells lose their viability in less than three days after collection, but resistant bacteria such as *B. subtilis* spores can retain viability on the filter for a

much longer time (Wang et al. 1999). As previously mentioned, the complex structure of bacterial spores protects cellular components by developing antimicrobial resistance, and low concentrations of chemical germinants can cause the spores to germinate (Moir et al. 2002). A study on the killing mechanism of spores by chemicals used in decontamination procedures demonstrated that spores can germinate even after they are treated with nitrous acid, while those treated with Betadine™ containing 1% available iodine do not germinate (Tennen et al. 2000). Our study demonstrates such a benefit of incorporating iodine with filtration for biocidal applications.

Surviving microorganisms on filters can re-entrain into the air passing through the filter medium, which has been reported in several studies (Qian et al. 1998; Wang et al. 1999; Rengasamy et al. 2004). A study on the reaerosolization of bacteria and solid particles from N95 respirators observed that the reaerosolization of particles smaller than 1 μm is insignificant (< 0.025%). Reaerosolization of larger aggregates of bacteria or bacteria attached to large inert particles, however, is significant at the same reaerosolization velocities, which correlate with violent sneezing and coughing, and at low (22%) RH (Qian et al. 1997). The reentrainment of fungal spores was higher than that of KCl particles due to disaggregation of fungal spores. Moreover, the rate is different among various fungal spore species depending on the surface structure (Jankowska et al. 2000). The present study showed very low extraction ($6.9\times10^{-4} \pm 1.6\times10^{-4}$) by vortexing. This value is much lower than that reported in a prior study for *B. subtilis* from polycarbonate filters, where the vortexing method exhibited extraction efficiency of 85% (Wang et al. 2001). In other words, the spores were securely trapped in the filter matrix of our test filters, resulting in inefficient extraction. This phenomenon is supported by the SEM images shown in Figure 2-4. The bacterial spores are attached to the fibrous surface due to van

der Waals forces. For an electret filter, the electrostatic interaction between the positively charged resin surface and negatively charged microorganisms further enhances the attachment. These two forces are weakened when water is present. Therefore, detachment is expected to be faster in water than in air. However, even with vortexing to enhance the dislodging, the spores were still trapped securely, implying that reaerosolization from such an electret filter in air will be low.

From a practical application perspective, the resin filter material without iodine treatment is an effective medium to trap the relatively large bacterial spores with negligible reaerosolization. In both iodine-treated and untreated filters, negatively charged bacterial spores are influenced by attractive force with the positive resin surface and repulsive force due to negatively charged functional groups on the filter medium. Specifically, the resin surface and the iodide ions remaining after depletion of iodine molecules from triiodide have similar attractive and repulsive force as the untreated filter. Therefore, both iodine-treated and untreated filters presumably have similar retention of the bacterial spores, suggesting that the filter medium that is depleted of iodine over time can still serve as an effective medium trapping the spores.

It should be noted that the efficacy of a biocidal filter is observed for bacterial spores collected on the filter, which are exposed to iodine disinfectant in the filter for several hours. The separate question about inactivation of penetrating bacterial spores by interactions during the short penetration time (*i.e.*, 14 ms) is not addressed by this experiment because the number of penetrated bacterial spores is too low to distinguish a biocidal effect. Further evaluation of smaller microorganisms, which exhibit higher penetration, is warranted to generalize the assessment of a biocidal agent on the penetrating microorganisms and application to a wide range of biological agents.

One important thing considered in the use of the biocidal filter is the health effect of the incorporated antimicrobial agent. Since iodine vapor irritates mucous membranes and adversely affects the upper and lower respiratory system, its inhalation can cause coughing and tightness in the chest (Cameron 2002). The iodine concentration in the air passing through the iodine-treated filter is as low as the detection limit of the analytical method, which is 0.004 mg/m^3 (OSHA 1994). It is much less than the 8-hr Time Weighted Average-Threshold Limit Value (TWA-TLV) of 1 mg/m^3, which is the level below which a worker is expected to have no adverse health effect resulting from chronic exposure (OSHA 2000).

In conclusion, both the iodine-treated and untreated filter media present effective approaches to the removal of bacterial spore aerosols. They achieve high viable removal efficiency without increasing pressure drop by incorporating iodine as a disinfectant into the filter medium. Furthermore, the deactivation of the collected bacterial spore aerosols is enhanced by the iodine-treated filter compared to the untreated filter before the filter medium loses significant amount of iodine due to sublimation and dissolution.

Table 2-1. Removal efficiency of the iodine-treated and untreated filters for bacterial spore aerosols at various environmental conditions

Environmental conditions	Test Filters	Trial No.	Challenge (CFU)	Penetration (CFU)	Removal efficiency (%)
Room Temp. Low RH[†]	Iodine-treated filter	1,5,7,9,10, 11,13,14,15	4.9×10^4 – 9.8×10^4	No	> 99.9980
		2	9.5×10^4	1	99.9989
		3	1.1×10^5	2	99.9981
		4	8.7×10^4	1	99.9988
		6	8.0×10^4	1	99.9988
		8	6.5×10^4	1	99.9985
		12	5.8×10^4	1	99.9983
	Untreated filter	1,2,4,6,7,9, 11,12,14	4.2×10^4 – 8.7×10^4	No	> 99.9976
		3	6.4×10^4	1	99.9984
		5	6.7×10^4	1	99.9985
		8	6.3×10^4	2	99.9968
		10	5.6×10^4	2	99.9965
		13	5.9×10^4	1	99.9983
		15	6.1×10^4	1	99.9984
Room Temp. High RH[‡]	Iodine-treated filter	1,2,3	7.3×10^4 – 8.1×10^4	No	> 99.9986
		4	8.0×10^4	1	99.9987
High Temp. High RH[‡]	Iodine-treated filter	1,3,4	8.7×10^4 – 9.3×10^4	No	> 99.9989
		2	9.0×10^4	1	99.9989

Table 2-2. Survival fraction of bacterial spores on both filters at various environmental conditions

Environmental Conditions	Test Filters	Average ± S.D
Room Temp. Low RH*	Iodine-treated filter	$6.9\times10^{-4} \pm 1.6\times10^{-4}$
	Untreated filter	$2.5\times10^{-3} \pm 1.4\times10^{-3}$
Room Temp. High RH	Iodine-treated filter	$5.1\times10^{-3} \pm 5.5\times10^{-3}$
High Temp. High RH	Iodine-treated filter	$8.3\times10^{-3} \pm 5.8\times10^{-3}$

* Significant difference between the result of iodine-treated filter and untreated filter

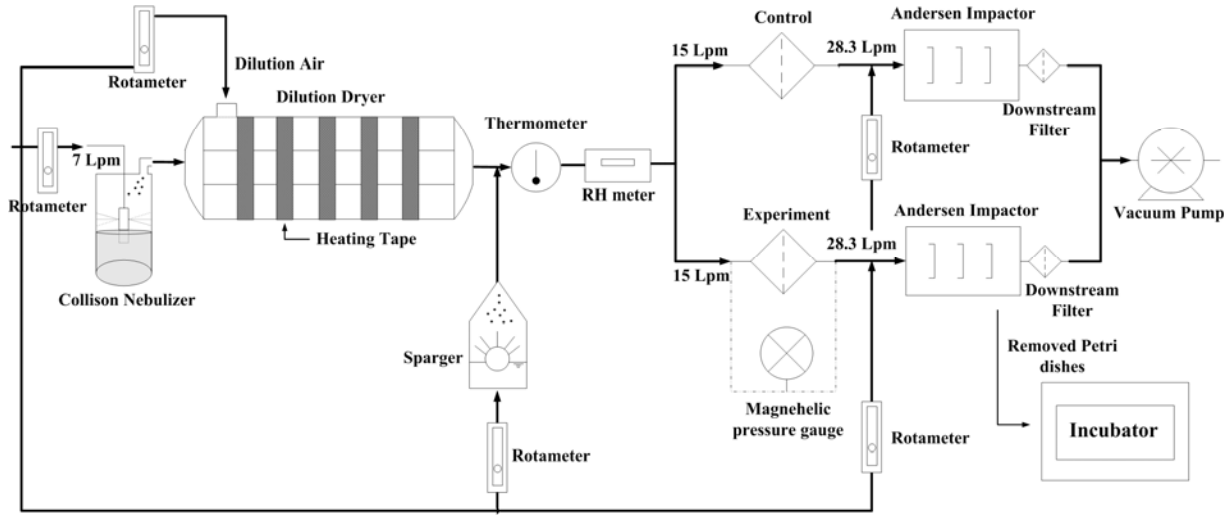


Figure 2-1. Experimental setup for bacterial aerosol system

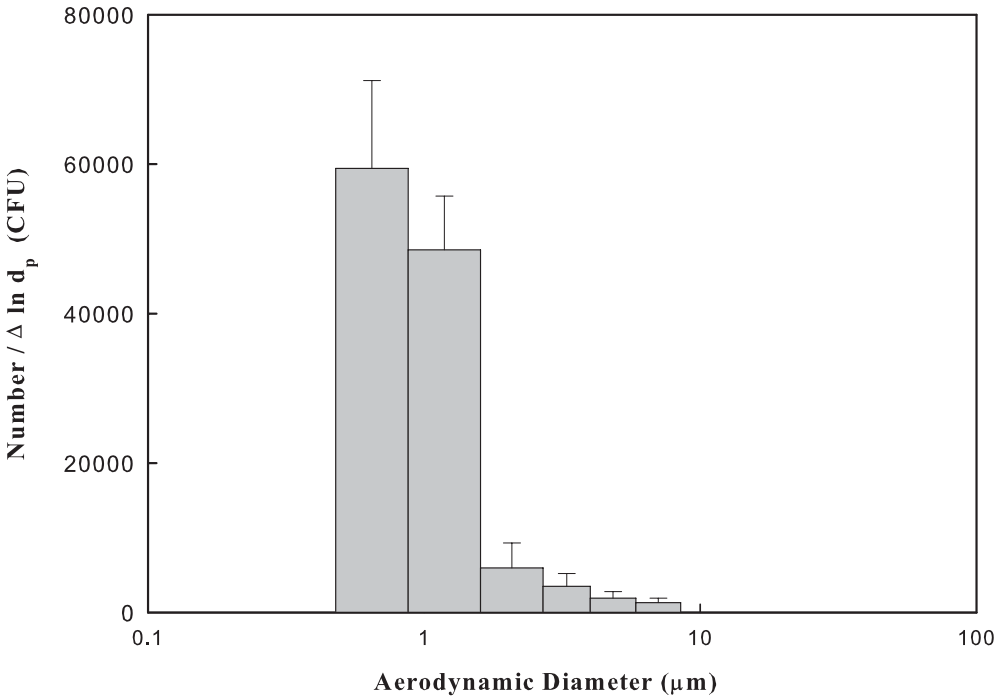


Figure 2-2. Particle size distribution of entering bioaerosols

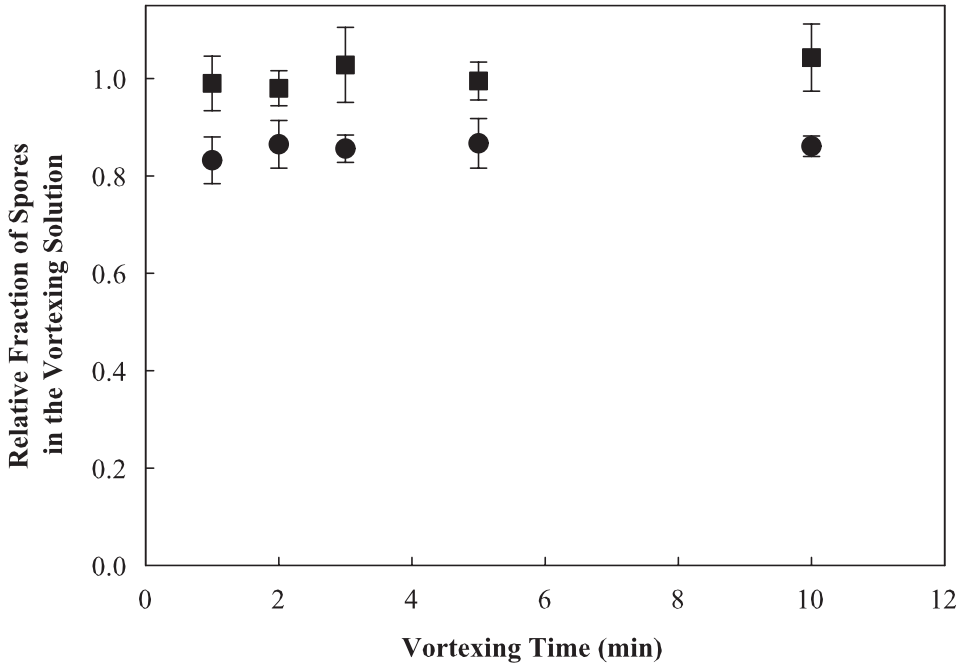
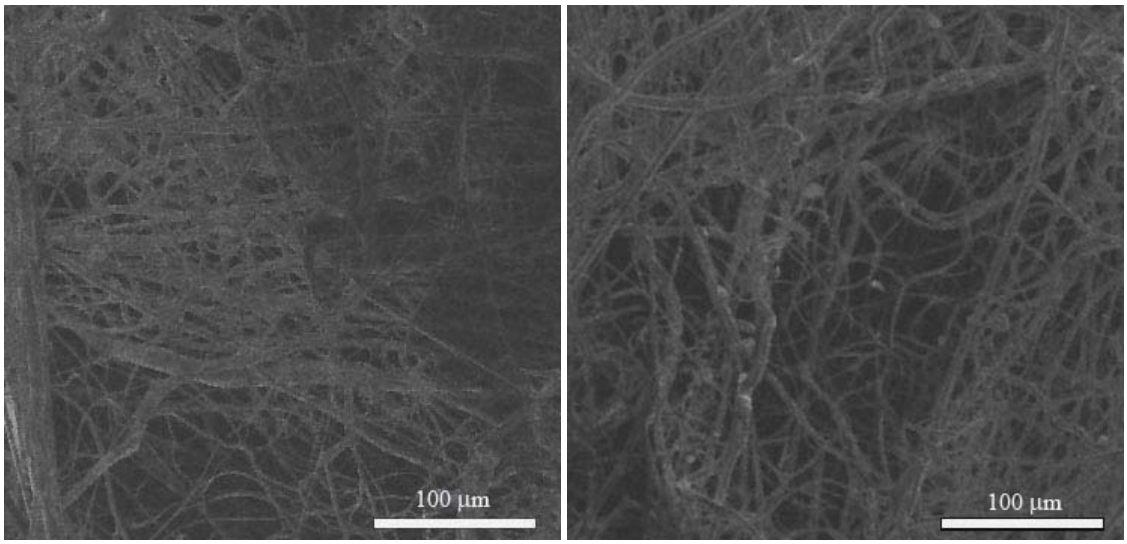


Figure 2-3. Relative fraction of spores in the vortexing solution of the clean iodine-treated and untreated filters



A B

Figure 2-4. SEM images of the filters at 100X. A) Unused iodine-treated filter. B) Iodine-treated filter after vortexing experiment

CHAPTER 3
ASSESSMENT OF IODINE-TREATED FILTER MEDIA FOR REMOVAL AND INACTIVATION OF MS2 BACTERIOPHAGE AEROSOLS

Objective

The objective of the study in this chapter was to evaluate an iodine-treated filter medium for removal and inactivation of viral aerosols under various environmental conditions and explore inactivation mechanisms of the filter. Physical removal efficiency (PRE), viable removal efficiency (VRE), pressure drop, I_2 concentration in the impinger medium, and the infectivity of viruses collected on the iodine-treated filter were investigated and compared with those of an untreated filter. The inactivation mechanism proposed earlier for the iodine-treated filter was examined by measuring VRE downstream of the filter using various collection media that were inert, moderately reactive and aggressively reactive to I_2. Furthermore, a second possible source of inactivation mechanisms was considered—I_2 released from the filter and transported to the impinger where the inactivation was hypothesized to occur.

Materials and Methods

Test Filters

Samples of the iodine-treated (polyester–cotton, 125 g/m^2 triiodide resin, Safe Life Corp., San Diego, CA, USA) and untreated (polyester–cotton, Safe Life Corp., San Diego, CA, USA) filter media, both as flat sheets 1 mm thick, were tested as discs of 47– mm diameter. The information on the preparation of an iodine-treated filter is described in Chapter 2. The I_2 concentration was measured to be 0.004 mg I_2/m^3.

Test Microorganisms

MS2 bacteriophage (ATCC® 15597-B1™) was selected as a representative virus aerosol. In the selection of a model virus, its resistance to antimicrobial agents should be considered because resistance varies from one virus to another (Berg et al. 1964; Sobsey et al. 1990). MS2

is a non-enveloped, icosahedron-shaped, single-stranded RNA with a single-capsid size of 27.5 nm, and it infects only male *Escherichia coli* (Prescott et al. 2002). MS2 has been used as a surrogate for small RNA enteroviruses pathogenic to humans because they both have no lipid component surrounding the protein coat and are considered to have similar resistance (Aranha-Creado and Brandwein 1999; Brion and Silverstein 1999). Freeze-dried MS2 was suspended with filtered deionized (DI) water to a concentration of 10^8–10^9 plaque forming units (PFU)/mL as the virus stock suspension and stored at 4 °C.

Experimental System and Conditions

The experimental set-up for testing the removal efficiency of filters is shown in Figure 3-1. Seven Lpm (liters per minute) of dry, filtered compressed air was passed though a six-jet Collison nebulizer (Model CN25, BGI Inc., Waltham, MA, USA) to aerosolize the viral suspension. The virus concentration in the Collison nebulizer was 10^5–10^6 PFU/mL and was prepared by diluting 0.10 or 0.20 mL of virus stock suspension in 50 mL of sterile DI water. The aerosolized particles were dried with filtered compressed air in a 2.3-L glass dilution dryer. A flow rate of 8 Lpm, which corresponds to a face velocity of 14.2 cm/s, was used for each stream (*i.e.*, control and experimental) and controlled by a calibrated rotameter. Based on the velocity, flight time through the 1-mm filter is estimated to be 0.007 seconds. This face velocity, used by Safe Life Corp., corresponds to certification testing of 100 cm^2 media (Di Ionno and Messier 2004) against the 85–Lpm flow rate specified by the National Institute for Occupational Safety and Health (NIOSH 2005). Pressure drop across each filter was monitored with a Magnehelic gauge measuring 0–2491 Pa and recorded every 30 minutes. The viral aerosols entering and penetrating the test and control filters were collected in an AGI-30 impinger (Ace Glass Inc., Vineland, NJ, USA) containing 20 mL of sterile phosphate buffered saline (PBS). The collection

medium in each impinger was replaced by fresh solution every 30 mins and assayed to determine the virus concentration by using suitable dilution to an adequate count (*i.e*., 30–300 PFU). The procedures for preparing plaque assay medium are presented in Appendix B. Five 2-hr trials were conducted, and thus total evaluation time was 10 hrs.

Since I_2 and HOI are disinfective forms, an increased VRE of the iodine-treated biocidal filter at high temperature and increased relative humidity (RH) was hypothesized due possibly to iodine's sublimation and to increased dissolution through the hydrolysis of I_2 to HOI. Therefore, various environmental conditions were considered: room temperature (23±2 °C) and low relative humidity (35±5%, RT/LRH); high temperature (30±2 °C) and LRH (HT/LRH); RT and medium RH (50±5%, RT/MRH). Because the maximum inactivation of MS2 aerosolized from 0.1*M* NaCl was reported to occur at 75% (Trouwborst and de Jong 1973), RHs below this level were considered. Temperature and RH were adjusted by wrapping the dilution dryer with a heating jacket and adding dry or humid dilution air to the system.

Removal Efficiency

Removal efficiency of viral aerosols by the test filters can be expressed both as PRE and as VRE. The particle size distribution (PSD) of the aerosols entering and penetrating the test filters was measured by using a Scanning Mobility Particle Sizer (SMPS; Model 3936, TSI Inc., Shoreview, MN, USA) and the PRE was determined by using Eq. (3-1).

$$\text{PRE (\%)} = \left(1 - \frac{N_P}{N_E}\right) \times 100 \tag{3-1}$$

where N_E is the number of particles entering the filter and N_P is the number of particles penetrating the filter.

The VRE depends on the infectivity of viruses collected in the impingers. The VRE was determined by counting plaques on each Petri dish from both control and experimental impingers, and calculated according to Eq. (3-1). In calculating the viral concentration, a dilution factor was used, which depends on the number of transfers of the impinger solution. Thus, the viral concentration in the impinger, C_v (*PFU*/mL), was determined by using Eq. (3-2).

$$C_v = \frac{PFU}{10^{-n} \times V} \tag{3-2}$$

where *PFU* is the number of plaque-forming units, *V* is the volume of diluted solution, and *n* is the dilution factor. The final mean viral concentration was determined by averaging all values in each dilution.

Inactivation Mechanism of the Iodine-Treated Biocidal Filter

Two possible inactivation mechanisms of the iodine-treated filter were considered: (1) inactivation of viruses downstream of the filter by reaction with I_2 released from the filter and (2) direct transfer of I_2 during near contact as viral aerosols pass through the iodine-treated filter.

- Sublimation and Dissolution of Iodine Molecules Released from the Filter

To investigate the effects of iodine released from the iodine-treated filter, filtered clean air passing the test filter at various environmental conditions was drawn into impingers containing a viral suspension of known concentration. The virus in the experimental impinger might lose its infectivity due both to the operation of the impinger (*e.g.*, swirling effect and reaerosolization) and to the action of I_2. Meanwhile, the infectivity of viruses in the control impinger will be affected only by the operation of the impinger. Therefore, by comparing the results of the control and the experimental impingers, the loss of virus infectivity by the operation of the impinger was excluded.

How I_2 disinfects virus in the impinger was studied by using sodium thiosulfate solution to quench the reactivity of I_2 available in the impinger. The same experimental procedure described previously for sublimation and dissolution of I_2 was followed except that the impinger medium was replaced by a 0.1*M* solution of sodium thiosulfate. Thiosulfate anion ($S_2O_3^{2-}$) reacts stoichiometrically with I_2 and reduces it to iodide, which is not virucidal (Berg et al. 1964).

- Transfer of I_2 to Viral Aerosols

To investigate the inactivation mechanism of direct transfer of I_2 to viral aerosols, the effect of sublimation and dissolution of I_2 released from the iodine-treated filter should be excluded. The use of thiosulfate solution has a limitation in this exclusion because it can react both with I_2 existing free in the impinger solution and with I_2 residing on the MS2. Therefore, a halogen-demanding substance—bovine serum albumin (BSA)—was used, which consumes free I_2 in the impinger solution but competes less aggressively than thiosulfate for I_2 on the MS2. The capacity of BSA to consume all of the I_2 released from the filter was predetermined by using the same experimental configuration for sublimation and dissolution of I_2 except that the impinger contained 0.3%, 3% and 6% BSA and a virus suspension of known concentration. The filtration experiment was then performed using the selected concentration. Viral aerosols were delivered as challenges to the iodine-treated filter and collected in both control and experimental impingers for 1, 5, 10, and 15 mins. The MS2 in the experimental impinger was compared to the penetrating MS2. For comparison, the same experiment was performed with thiosulfate solution as the collection medium of the impinger for 15 mins.

Infectivity of Viruses on the Filter

After 10 hrs of removal efficiency experiments, the test filters were retrieved from the filter holder in the experimental system and subjected to a vortex mixer (Model M16715,

Barnstead, Dubuque, IA, USA) to investigate the infectivity of viruses collected on the filter. The filter was immersed in 40 mL of sterile DI water in a 250-mL beaker and vortexed for a designated time (*i.e.*, 0, 1, 3 and 5 min) to investigate the optimal extraction time. The vortexing solution was assayed to determine the infectivity of viruses and the number of viruses (N_v) was determined by using Eq. (3-3).

$$N_v = \frac{PFU}{10^{-n}} \times \frac{V_1}{V_2} \tag{3-3}$$

where V_1 is the volume of extraction fluid and V_2 is the volume of original or diluted suspension assayed with host cells. The total infectivity of extracted viruses was calculated by averaging the results at all vortexing times because the number of extracted viruses at each designated vortexing time was found to be similar. The extracted fraction—the ratio of the infectivity count in the extraction solution to the total viruses collected on the filter—was used to compare the result of the iodine-treated filter with the untreated filter.

Effects of Free Iodine Molecules

In an aqueous suspension for the vortexing experiment, the resin surfaces are expected to release I_2 that can inactivate viruses. This reaction raises a question whether viruses lose their infectivity in the extract solution due to the free I_2 residual or on the filter. To investigate this question, the solution after vortexing a clean iodine-treated filter for a designated length of time (0, 1, 3 and 5 min) was inoculated with a virus suspension of known concentration. Because it took 15 minutes to finish the vortexing experiment including dilution and assay, the infectivity of virus in the mixed suspension was analyzed after 15 minutes of exposure to the free I_2 in the suspension. The I_2 concentration in the vortexing solution was analyzed by the DPD (*N*, *N*-diethyl-*p*-phenylenediamine) colorimetric method adapted from *Standard Methods for the Examination of Water and Wastewater 4500-CI G* (APHA 1995). Ten mL of solution vortexed

with the iodine-treated filter was analyzed at 530 nm by using a DR/4000 V Spectrophotometer (Hach, Loveland, CO, USA). I_2 in the solution reacts with DPD to form a pink color, the intensity of which is proportional to the total I_2 concentration (Hach 2003). The effect of vortexing alone on the infectivity of viruses was also investigated by following the same vortexing procedure with a virus suspension of known concentration.

Results

Physical Removal Efficiency and Pressure Drop

The PRE of the test filters was determined by comparing the PSDs of the aerosols entering and penetrating the test filters as shown in Figure 3-2. The PSD of the aerosols entering the test filters showed its mode at approximately 25 nm. As a baseline, sterile DI water without virus was aerosolized from the nebulizer and the PSD of that was measured, defining the background noise. Therefore, the PSD of the aerosols above the noise level in the window from 9.82 to 162.5 nm was considered for the calculation. The PRE (mean ± SD) of the iodine-treated and untreated filters for this size range were 41±3 % and 39±2 %, respectively. Statistical evaluation of the two values by a one-tailed student's *t*-test indicated that the difference was insignificant (p-value > 0.05).

The initial pressure drop of the test filters was around 50–100 Pa and the variation in pressure drop during the entire experiment was negligible. This value is much less than the inhalation and exhalation resistances of the respirator certified by NIOSH, which cannot exceed 343 Pa and 196 Pa, respectively (CFR 2002). No significant difference in the pressure drop between the iodine-treated and untreated filters was observed.

Viable Removal Efficiency

The VRE of the test filters was calculated by analyzing the infectivity of viruses collected on both control and experimental impingers for challenging and penetrating viruses from the filter. The result is presented as an average of five 2-hr experimental runs for each filter indicated as No.1 and No. 2 in Table 3-1 (raw data are available in Appendix C). As shown, the iodine-treated filter presented a significantly higher VRE than that of the untreated filter (p-value < 0.05) at various environmental conditions. At HT/LRH, a significantly higher value of the iodine-treated filter than that of the other conditions was observed, according to one-way ANOVA (p-value < 0.05), due to increased release of I_2 from the filter. Meanwhile, the difference between RT/LRH and RT/MRH was not significant (p-value > 0.05), indicating that the release of HOI into air due to the hydrolysis of I_2 at increased RH is negligible.

Inactivation Mechanisms of the Iodine-Treated Biocidal Filter

The effect of sublimation and dissolution of I_2 was investigated by using the impingers containing a virus suspension of known concentration either in the PBS or sodium thiosulfate solution. As shown in Table 3-2, no surviving virus was detected in the experimental impinger until $> 10^4$ PFU in the PBS was added to the impingers. As the virus concentration in the impinger increased, the number of surviving viruses also increased. Meanwhile, the survival fraction of viruses in the thiosulfate solution was much higher than that in the PBS. Most viruses suspended in the thiosulfate solution survived in the experimental impinger due to quenching by reaction with thiosulfate of the I_2 released from the iodine-treated filter and/or I_2 transferred to viral aerosols. Hatch et al. (1980) proposed spontaneous dissociation of I_2 from the polyiodide–resin complex as one of three possible inactivation mechanisms of their iodinated resin filter in water treatment. In another study (Marchin et al. 1983), acquisition of I_2 by a cyst during passage through an iodinated resin column was hypothesized. The authors observed that

cysts regained viability due to reduction of I_2 by thiosulfate solution for up to 3 mins. A more recent study (Brion and Silverstein 1999) reported reversal of MS2 inactivation after a few minutes (< 5 mins) of iodine treatment by adding 0.3 % BSA. It must be noted that these studies were performed in water, so their applicability to inactivation mechanisms of iodine in air treatment is uncertain.

In the experiments measuring I_2 demand of BSA, various concentrations of BSA were evaluated. As shown in Table 3-2, the survival fractions of MS2 in the experimental impinger having 3 % and 6 % BSA were similar to those in the control impinger (~0.95). The result indicates that both 3 % and 6 % BSA solutions contain sufficient protein to exhaust I_2 released from the filter and thus isolate MS2 in the experimental impinger from inactivation by I_2 in solution. The history of iodination of albumins suggests significant dependence on conditions. Muus et al. (1941) reported rapid uptake of 15 wt% iodine by horse serum albumin (HSA) from ~0.2*N* I_2/KI in aqueous ethanol and Shahkrokh (1943) added 8 wt% iodine to HSA with a similar concentration of I_2/KI in water. Hughes and Straessle (1950) incorporated 30 molar equivalents of iodine into human serum albumin in 0.1*N* aqueous I_2, converting 70% of L-tyrosine residues into diiodotyrosine. Small-scale preparations adding chloramine-T to similar concentrations of $K^{131}I$ in water achieved fast and efficient incorporation of the small amount of ^{131}I into human growth hormone (Greenwood et al. 1963), BSA (Opresko et al. 1980) and BSA microspheres (Smith et al. 1984). Lee and Ellis (1991) proposed the reaction with iodine solutions as a method to visualize serum albumins on polyacrylamide gels. However, Shahkrokh (1943) also showed that the extent of reaction of HSA with I_2 falls off rapidly with decreasing concentration and Portenier et al. (2001) reported that an equimolar amount of BSA did not suppress the bactericidal activity of a 0.2% solution of I_2/KI.

In the experiments in which aerosolized MS2 penetrated the iodinated filter, collection in a medium containing thiosulfate effectively neutralized all of the iodine released, whether displaced and captured or dissociated, as no decrease in viable penetration was observed (shown in Figure 3-3). In contrast, a similar experiment in which the penetrating particles and free iodine were collected in 3% BSA medium, showed that half the penetrating MS2 virions were inactivated initially and a moderate increase in survival was seen after 10 minutes. The initial observation is consistent with the mechanism proposed by Ratnesar–Shumate et al. (2008) because the data in Table 3-2 show that the capture medium is able to consume all of the free iodine coming off the filter. Thus, at least half of the MS2 viral particles penetrating the filter in this experiment appear to have acquired and bound a lethal dose of I_2 as they traversed the iodine-treated filter. The distinguishable increase of surviving MS2 at 10 mins of collection time parallels a delayed reactivation of MS2 observed in aqueous iodine solutions (Brion and Silverstein 1999), and it is tempting to conclude that the deactivation processes in water and in this system are similar after iodine has been transported to the virion. However, some combination of direct transfer of I_2 from the filter plus dissociation of I_2 from weaker binding sites on penetrating particles reproduces their general conditions and appears to cause almost half the observed inactivation of viral aerosols penetrating the iodine-treated filter and collected in PBS medium. After submission of a manuscript describing this effect, Triosyn Corp. (Messier 2009) disclosed data showing a threshold for inactivation of MS2 and of *Staphylococcus aureus* at 0.5~0.6 ppm I_2 in PBS medium, which is consistent with results presented herein and defines a boundary condition to anticipate significant interference by dissolved iodine. We then verified that the data reported by Heimbuch and Wander (2006) and by Heimbuch et al. (2007) were measured under conditions that inactivation by free I_2 did not contribute significantly.

Eninger et al. (2008a) collected MS2 aerosols penetrating an iodinated medium onto gelatin-coated plates, which they washed out into water and plated in a plaque assay. They observed no kill of MS2 and concluded that the treatment was ineffective. However, their observation of no inactivation by iodine during the steps of their workup that were executed in water shows clearly that the overwhelming excess of protein in their collection surface consumed all of the iodine displaced, released or captured from the iodinated medium. Whereas their experiment thus does not support the conclusion that the treatment is inactive, in the absence of measurements of I_2 concentrations in the impingers we can make no quantitative statement about the relative importance in our data set of these potentially competing processes for inactivation. However, we note that, even though sufficient I_2 is released to confound the environment in the impingers, the airborne concentration of I_2 released from the filter was much less than the 8-hr Time Weighted Average-Threshold Limit Value (TWA-TLV) of 1 mg/m^3, the level below which a worker is expected to have no adverse health effect resulting from chronic exposure (OSHA 2000). Hence, whatever activity is present is realistically available for use in respiratory protection.

Effects of Free Iodine Molecules and Extracted Fraction

To account for the effect of free iodine in the extract solution, the infectivity of viruses mixed with the vortexing solution from a clean iodine-treated filter after each designated vortexing time was analyzed and expressed as survival fraction (C_S/C_I, C_S: surviving MS2, C_I: initial MS2 in the suspension). The average value of the survival fraction at all vortexing times, 0.17 (*i.e.*, 83% attenuation), was used to correct the value for the infectivity of viruses collected on the filter. As presented in Table 3-3, the I_2 concentration in the vortexing solution measured by the DPD colorimetric method was around 1.0 mg/L I_2. Some I_2 was released from the iodine-

treated filter before the start of the vortexing procedure, designated as "0" vortexing time. No further increase of I_2 extraction from the filter by increasing vortexing time was observed.

The infectivity of viruses collected on the filter is presented as the extracted fraction (C_E/C_C, C_E: MS2 extracted from the filter, C_C: MS2 collected on the filter). *Cc* for the iodine-treated filter was determined from the VRE of the untreated filter because both iodine-treated and untreated filters had a similar PRE. The effect of vortexing on the viruses was negligible because the infectivity of viruses vortexed at various times did not have observable variation. Table 3-4 presents both observed and corrected values of the extracted fraction. The corrected values were determined by dividing the observed values by the survival fraction (0.17) to consider the effects of free I_2. As shown, no significant difference in the corrected extracted fraction between iodine-treated and untreated filters at the same environmental condition was exhibited (p-value > 0.05). Both iodine-treated and untreated filters tested at MRH showed the lowest value among the survival fractions presumably due to the sensitivity of MS2 to the MRH (Dubovi and Akers 1970). The lower values of free I_2 from the iodine-treated filter tested at HT/LRH and RT/MRH than that at RT/LRH indicate measurable loss of I_2 from the iodine-treated filter. Although the filter constantly experienced loss of I_2, it was observed that the efficacy of the iodine-treated biocidal filter did not deteriorate during 10 hrs of experiment.

After vortexing, one tested filter and one unused iodine-treated filter were examined under a scanning electron microscope (JSM-6330F, JEOL Ltd., Tokyo, Japan). As shown in Figure 3-4, abundant particles were observed in the tested filter compared to the unused filter.

Discussion

Intrinsic differences in test methods complicate comparison of PRE and VRE values measured for test filters. The PRE was measured for ultrafine particles (*i.e.*, 9.82 to 162.5 nm), whereas the VRE was measured over the entire particle size range generated from the nebulizer.

Even if the PRE for the entire particle size range is calculated by particle counting, its value will still be different from the VRE because of aggregation of virus aerosols and fewer counts of viable virus available for disaggregation in smaller particles than in bigger particles. A viral aggregate is measured by the particle counter as one particle, but it can be assayed as several viruses after collection in the impinger because of dispersion in the collection medium. The number of viable viruses in a big particle is larger than that in an ultrafine particle; thus, the contribution of larger particles collected in the impingers to the infectivity results will be much greater than that of ultrafine particles. This effect was observed in a prior study (Hogan et al. 2005), which reported that the probability of containing viable viruses increases with the size of particles from MS2 suspension .

In the experiment for sublimation and dissolution of I_2, the observed increase of survived viruses as virus concentration in the impinger increased is presumably due primarily to exhausting the supply of I_2 but might also include some shielding effect of aggregated/encased viruses if the aggregate persists in the impinger. Berg et al. (1964) reported that deactivation of viruses by iodine follows first-order reaction kinetics, and thus reaction rates of iodine with viruses depend on the number and availability of vital sites on the virion. They mentioned a lagged deactivation curve of iodine due to virus clumping and the necessity of time for virus clumping to be separated. A study of the survival of viral particles in aqueous suspension irradiated with ultraviolet light demonstrated that virus survival was strongly dependent on the degree of aggregation among the viral particles (Galasso and Sharp 1965).

The SEM images of the tested filter in Figure 3–4 show that many particles still remained on the filter after extraction. One can argue that it is due to inefficient extraction of the vortexing process. However, the extracted fraction from glass fiber HEPA filters (162 ± 61) following the

same vortexing procedure was much higher, demonstrating that vortexing extraction was efficient for regular filter media (Li et al. 2008). High retention capability of the electret test filter can be a reason for the low extracted fraction due to electrostatic attraction between viral particles and filter media. In the same context, insignificant reaerosolization of the viruses from the test filters is expected. It should be noted that both iodine-treated and untreated filters presumably have similar retention of viruses. In the test filters, the negatively charged surface of viruses is influenced by an attractive force with the positive resin surface and repulsive force due to negatively charged functional groups on the filter medium. This property of the test filter implies that a filter medium that is depleted of I_2 over time can still serve as an effective medium for trapping viruses because it has the same attractive and repulsive forces as the untreated filter—the resin surface and by-product iodide ions remain after consumption of the iodine molecules from the triiodide ions.

The effect of iodine on the infectivity of MS2 collected on the iodine-treated filter is less certain than previously thought, because similar viable recoveries were observed for the iodine-treated and untreated filters; however, a strong virucidal effect of I_2 was observed in both the VRE of the iodine-treated filter and free I_2 residual experiments. This phenomenon can be explained by two possible reasons: (1) shielding effect of aggregated particles collected on the filter and (2) high retention capability of the test filters.

- Shielding effect

MS2 in suspension is vulnerable to iodine, because the virus is better dispersed in an aqueous medium, whereas in the air it can be aggregated or encased in other constituents of particles that protect it from iodine inactivation. This assertion is supported by the SEM images shown in Figure 3-4. Most particles observed in the tested filter are orders of magnitude larger

than a single naked MS2, which can be either the MS2 aggregates or substances with MS2 generated from the nebulizer suspension (virus stock suspension in the nebulizer contains milk proteins and organic molecules for virus preservation). Therefore, infectivity of MS2 can be shielded by the outer layer of the aggregates or by encasement in substances present in the nebulization medium. MS2 aggregation generated from the nebulizer, which is caused by hydrophobic interactions between neighboring protein capsids, has been observed by previous studies (Hogan et al. 2004; Balazy et al. 2006).

- High retention capability of the filter

The extracted fractions of both iodine-treated and untreated filters are significantly lower than the other regular filter media due to the expected high retention of particles on filter media resulting from electrostatic interaction between filter media and the charged surface of viral particles, as discussed earlier. It should be stated that this interaction will persist due to the inherent electret property of the resin-treated surface. Extracted values close to the detection limit can make the effect of iodine on the virus infectivity indistinguishable.

The control experiments carried out in this study with thiosulfate and BSA require that reported data generated in experiments collecting aerosols in aqueous media or on protein gels to measure the biocidal capacity of the iodine-treated filter be reexamined to consider the possibility of competition by dissolved I_2. Significant support for the previously proposed mechanism of charge-induced capture of iodine from bound triiodide is found in the observation of significant inactivation persisting in a BSA medium that was able to protect suspended virions from inactivation by impinging I_2 vapor. However, toxicity of iodine dissolved in the collection medium is likely to be a competing mechanism in warm environments, and the relative importance of each must be determined—or at least factored into the design and analysis

processes—at different conditions. Data from a different experimental approach might not encounter this uncertainty, and the assay is only a surrogate for the goal of the technology—enhancing respiratory protection against bioaerosol transmission of pathogens. Both the medium in the impinger and the protein gel have elements in common with respiratory mucosa, and for a person wearing individual protective gear, the time of transit from filter to mucosal surface is similar. However, competition by water and by proteins at the site of impaction might or might not behave the same as in the in vitro systems tested to date. So, the ultimate measure of enhancement of protection by surface-bound iodine—or any other reactive surface on filter fibers—will require data from animal exposure studies.

Table 3-1. Removal efficiency of the iodine-treated and untreated filters for MS2 aerosols at various environmental conditions in impingers containing phosphate buffered saline

Environmental conditions	Filter media		Virus Concentration (PFU/mL)*		Removal eff. (%)*
			Challenge	Penetration	
Room temp. (23±2 °C) Low RH (35±5%)	Iodine-treated	No.1	$1.0\times10^{5}\pm4.3\times10^{4}$	$5.3\times10^{2}\pm2.5\times10^{2}$	99.4±0.5
		No.2	$1.4\times10^{5}\pm5.8\times10^{4}$	$4.1\times10^{2}\pm3.4\times10^{2}$	99.7±0.4
	Untreated	No.1	$6.3\times10^{4}\pm5.6\times10^{4}$	$5.0\times10^{3}\pm4.4\times10^{3}$	92.4±1.8
		No.2	$3.7\times10^{4}\pm1.2\times10^{4}$	$3.3\times10^{3}\pm1.1\times10^{3}$	90.7±2.2
High temp. (30±2 °C) Low RH	Iodine-treated	No.1	$1.4\times10^{5}\pm7.0\times10^{4}$	N.D†	> 99.9995
		No.2	$3.0\times10^{4}\pm2.5\times10^{4}$	$3.2\times10^{0}\pm2.4\times10^{0}$	99.98±0.05
	Untreated	No.1	$3.3\times10^{5}\pm1.5\times10^{5}$	$1.6\times10^{4}\pm6.9\times10^{3}$	94.0±3.8
		No.2	$9.6\times10^{4}\pm3.0\times10^{4}$	$7.2\times10^{3}\pm2.7\times10^{3}$	91.4±4.8
Room temp. Medium RH (50±5%)	Iodine-treated	No.1	$2.4\times10^{4}\pm1.8\times10^{4}$	$6.7\times10^{1}\pm6.9\times10^{1}$	99.8±0.3
		No.2	$7.6\times10^{3}\pm3.2\times10^{3}$	$4.2\times10^{0}\pm8.8\times10^{0}$	99.8±0.8
	Untreated	No.1	$2.3\times10^{5}\pm2.4\times10^{5}$	$1.4\times10^{4}\pm1.3\times10^{4}$	93.4±2.1
		No.2	$1.0\times10^{5}\pm3.8\times10^{4}$	$8.9\times10^{3}\pm3.5\times10^{3}$	91.3±2.0

* The average (±S.D) of five 2-hr trials, † Not detected.

Table 3-2. The survived MS2 among various MS2 concentrations in the impingers with phosphate buffered saline, thiosulfate solution, and bovine serum albumin at various environmental conditions due to released iodine from the filter

Environmental conditions	Collection medium in the impinger	Virus count (PFU) in the impinger (Average ± SD)		Survival fraction*
		Control	experimental	
Room temp. (23±2 °C) Low RH (35±5%)	PBS †	5.6×10^{3}	0	0
		1.1×10^{4}	1.0×10^{2}	9.1×10^{-3}
		2.3×10^{5}	8.0×10^{2}	3.4×10^{-3}
	Sodium thiosulfate	$1.9\times10^{3}\pm4.9\times10^{2}$	$1.7\times10^{3}\pm4.9\times10^{2}$	$9.0\times10^{-1}\pm0.0$
High temp. (30±2 °C) Low RH	PBS	$6.3\times10^{3}\pm7.1\times10^{1}$	0	0
		$5.3\times10^{4}\pm8.5\times10^{3}$	$8.6\times10^{1}\pm3.4\times10^{1}$	$1.6\times10^{-3}\pm3.5\times10^{-4}$
		$2.1\times10^{5}\pm3.5\times10^{4}$	$5.0\times10^{2}\pm2.1\times10^{2}$	$2.4\times10^{-3}\pm4.9\times10^{-4}$
	Sodium thiosulfate	$1.4\times10^{3}\pm1.4\times10^{2}$	$1.2\times10^{3}\pm0.0\times10^{0}$	$8.5\times10^{-1}\pm7.1\times10^{-2}$
Room temp. Medium RH (50±5%)	PBS	$6.2\times10^{3}\pm2.5\times10^{3}$	0	0
		$6.5\times10^{4}\pm7.1\times10^{3}$	$4.9\times10^{1}\pm1.9\times10^{1}$	$7.3\times10^{-4}\pm2.1\times10^{-4}$
		$2.9\times10^{5}\pm1.4\times10^{4}$	$5.6\times10^{2}\pm4.2\times10^{1}$	$2.0\times10^{-3}\pm7.1\times10^{-5}$
	Sodium thiosulfate	$3.2\times10^{3}\pm2.5\times10^{3}$	$2.2\times10^{3}\pm1.5\times10^{3}$	$7.5\times10^{-1}\pm7.1\times10^{-2}$
	0.3 % bovine serum albumin	$1.6\times10^{3}\pm6.7\times10^{2}$	$9.1\times10^{2}\pm1.3\times10^{2}$	$5.9\times10^{-1}\pm1.7\times10^{-1}$
	3 % bovine serum albumin	$1.6\times10^{3}\pm7.4\times10^{2}$	$1.5\times10^{3}\pm6.1\times10^{2}$	$9.5\times10^{-1}\pm6.1\times10^{-2}$
	6 % bovine serum albumin	$1.9\times10^{3}\pm7.6\times10^{2}$	$1.7\times10^{3}\pm6.4\times10^{2}$	$9.5\times10^{-1}\pm4.3\times10^{-2}$

* PFU in the experimental impinger divided by PFU in the control impinger, † Phosphate buffered saline

Table 3-3. Iodine concentration (mg I_2/L)* in the vortexing solution at each vortexing time

Filter Media	Vortexing Time (min)			
	0	1	5	10
Iodine-treated filter	0.62±0.11	0.98±0.04	0.91±0.13	0.98±0.08

* The average measurement in triplicate

Table 3-4. Extracted fraction of MS2 on the iodine-treated and untreated filters at various environmental conditions

Environmental conditions	Filter media	Average ± SD		Iodine in vortexed solution (mg/L)
		Observed	Corrected*	
Room temp. (23±2 °C) Low RH (35±5%)	Iodine-treated	$3.4\times10^{-3}\pm1.4\times10^{-3}$	$2.0\times10^{-2}\pm8.4\times10^{-3}$	0.93±0.01
	Untreated		$3.6\times10^{-2}\pm3.4\times10^{-2}$	-
High temp. (30±2 °C) Low RH	Iodine-treated	$3.3\times10^{-3}\pm2.0\times10^{-3}$	$2.0\times10^{-2}\pm1.2\times10^{-2}$	0.575±0.007
	Untreated		$3.3\times10^{-2}\pm2.7\times10^{-2}$	-
Room temp. Medium RH (50±5%)	Iodine-treated	$1.2\times10^{-3}\pm5.0\times10^{-4}$	$6.9\times10^{-3}\pm2.9\times10^{-3}$	0.76±0.06
	Untreated		$5.5\times10^{-3}\pm9.2\times10^{-4}$	-

* The value was obtained by dividing the observed values by the survival fraction (0.17).

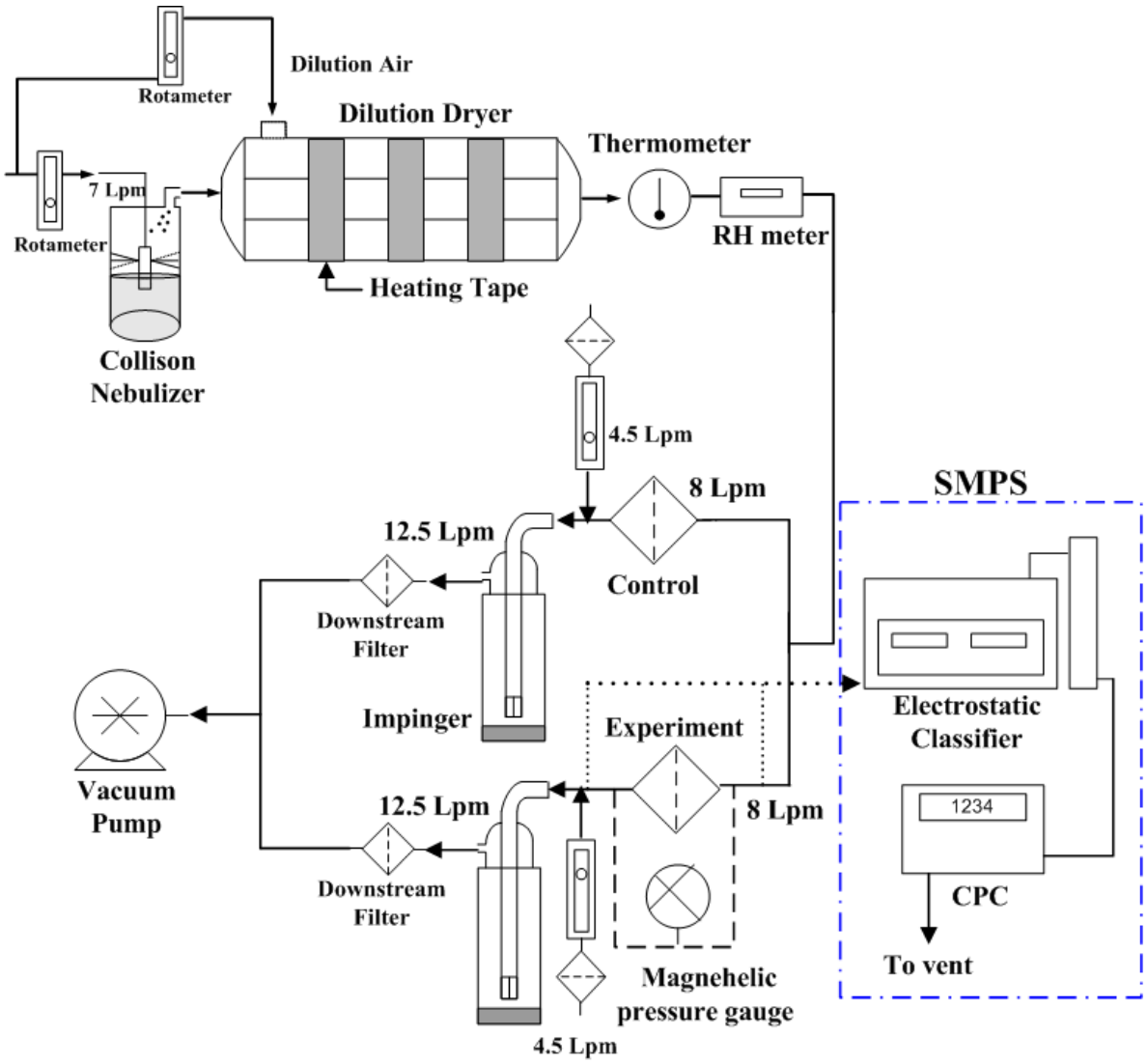


Figure 3-1. Experimental set-up. A) Viable removal efficiency. B) Physical removal efficiency of the test filters

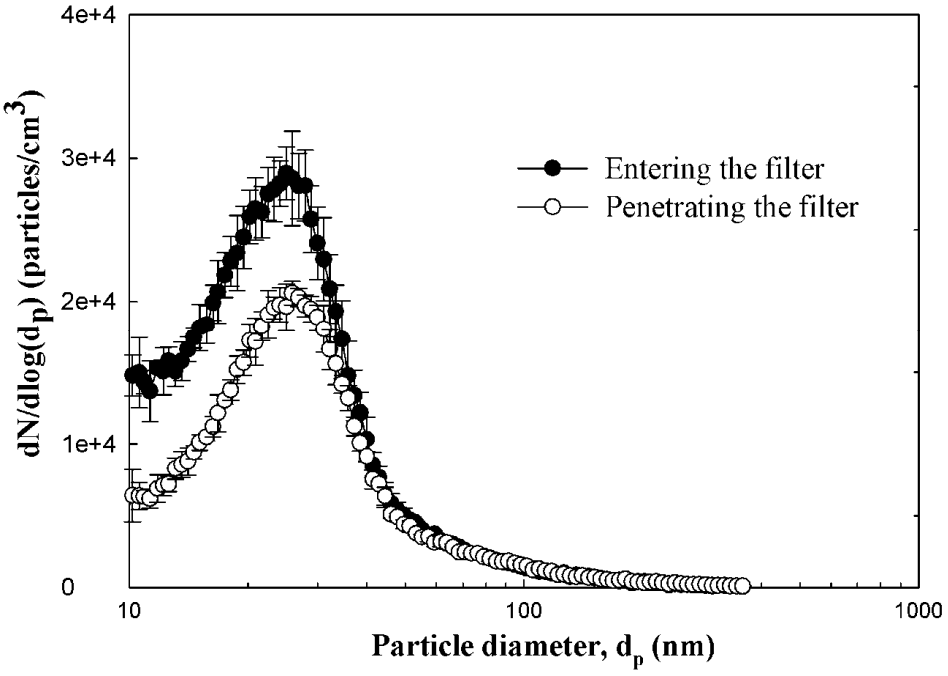


Figure 3-2. The number-based particle size distribution of aerosols entering and penetrating the filter at RT/LRH

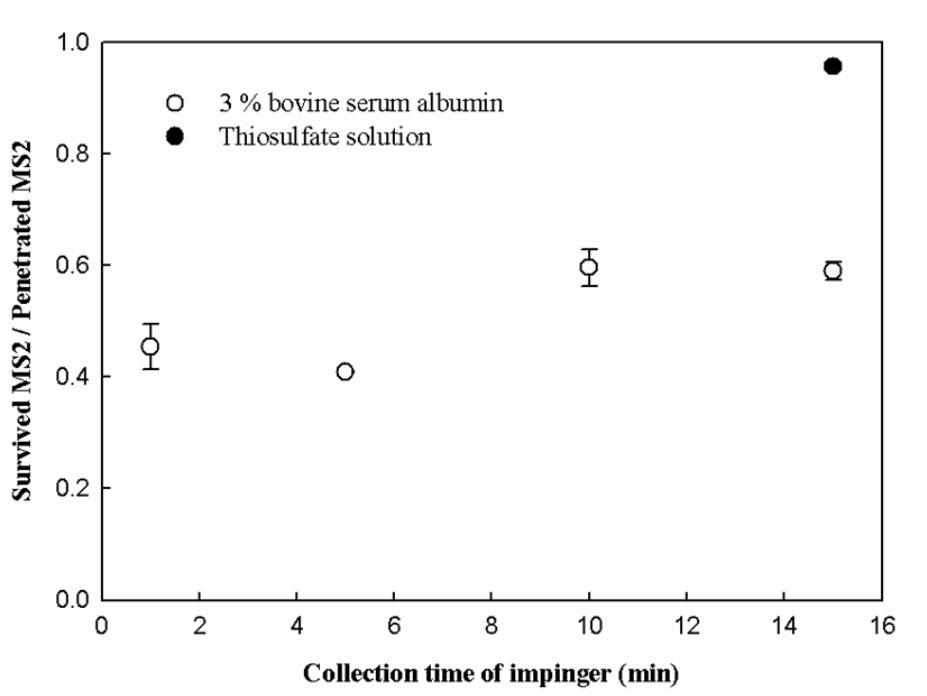
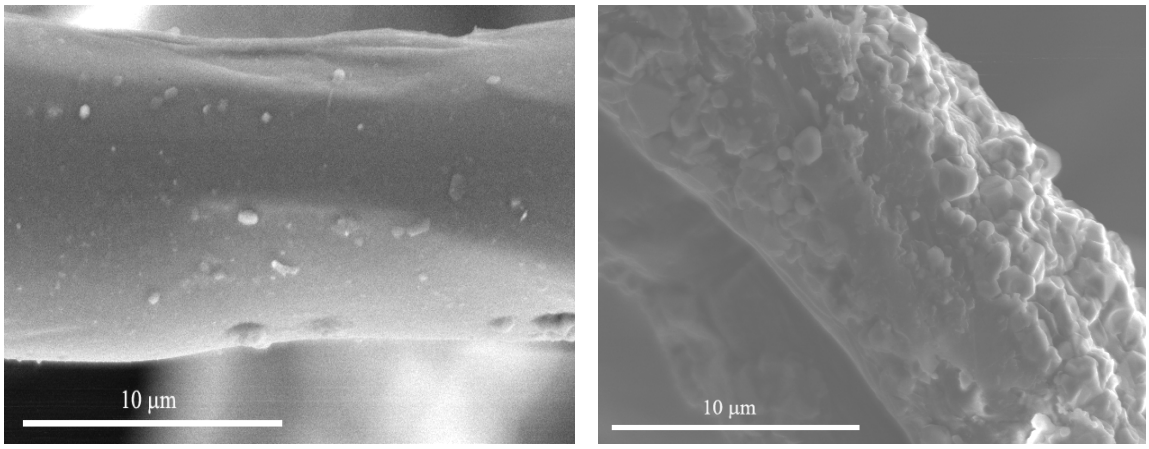


Figure 3-3. The survived MS2 aerosols among penetrated MS2 aerosols from the iodine-treated filter with thiosulfate solution and 3% bovine serum albumin as the collection medium of the impinger at room temperature and medium relative humidity



A B

Figure 3-4. SEM images of the filter at 2700X. A) Unused iodine-treated filter. B) Iodine-treated filter after vortexing experiment

CHAPTER 4
CHARACTERIZATION OF MS2 BACTERIOPHAGE AEROSOLS INFLUENCED BY RELATIVE HUMIDITY AND SPRAY MEDIUM

Objective

The objective of the study presented in this chapter was to characterize viral aerosols by investigating the number of infectious and total viruses, including infectious and non-infectious, in the ultrafine and submicrometer range. Relative humidity and spray medium from which viral aerosols were generated were factored into the investigation due to influence of those on the stability or infectivity of viral aerosols. The ultimate goal of this study is to provide information on how viruses are distributed and survive in aerosols under different environmental conditions. Such information is important to a wide range of applications such as development of protection method, respiratory deposition of viral aerosols and consequently risk assessment of airborne pathogens.

Materials and Methods

Test viruses

MS2 is a non-enveloped, icosahedron-shaped, single-stranded RNA with a diameter of 27 – 34 nm that infects only male *Escherichia coli* (Stokley et al. 1994; Prescott et al. 2002). MS2 has been used as a simulant for human pathogens of small RNA viruses such as Ebola virus, poliovirus, and rotavirus because of its similar physical characteristics including small size and simple structure. Because it is harmless to humans, economical and easy to culture and assay, MS2 has been used as a model microorganism in a number of studies: biological defense studies (Belgrader et al. 1998; Kuzmanovic et al. 2003; O'Connell et al. 2006), testing protection device against biological agents (Walker et al. 2004), and detection of microorganisms in environment (Alvarez et al. 2000). It consists of a 3,569–nucleotide genome encoding four

proteins–a coat protein, a maturation protein, a replicate subunit (or RNA replicase β chain), and a lysis protein – and 180 copies of the capsid protein (Fiers et al. 1976). The MS2 virus stock was prepared by suspending freeze-dried MS2 (ATCC® 15597-B1™), which contains a small amount of milk proteins and organic molecules for virus preservation, with filtered deionized (DI) water to a concentration of 10^9–10^{10} plaque–forming units (PFU)/mL and stored at 4 °C.

Experimental design

The experimental set-up to investigate the infectious and non-infectious viruses as a function of particle size is shown in Figure 4-1. The aerosols containing viruses were produced by a Collison nebulizer and dried in the dilution dryer to remove water content. The resultant aerosol had a polydisperse particle size distribution (PSD), which was characterized by using the scanning mobility particle sizer (SMPS), a device that operates as the combination of an electrostatic classifier with a long differential mobility analyzer (DMA) and a condensation particle counter (CPC), as shown in Figure 4-1 (A). Since the change of PSD over the entire generation is important, the PSD was monitored for 35 min, which was the time needed to conduct the experiment.

The voltage applied to the differential mobility analyzer (DMA) can be tuned to allow only aerosols of a specific size to exit the electrostatic classifier. The size–classified aerosols were subsequently collected in a BioSampler® (SKC Inc., Eighty Four, PA, USA) for 5 mins with a flow rate of 4.5 Lpm as shown in Figure 4-1 (B). The reason for using a flow rate lower than the standard one (*i.e.,* 12.5 Lpm) is to avoid significant reaerosolization from the impinger at the higher flow rate. Because Riemenschneider et al. (2009) reported insignificant reaerosolization (<1 %) over short sampling periods, 5 min of sampling time was selected. The samples in the BioSampler were then analyzed with a plaque assay method (Lee et al. 2009) by

inoculating host cells in the samples and polymerase chain reaction (PCR) to investigate infectious and total viruses, respectively.

Since the effect of RH on the stability or infectivity of viral aerosols was hypothesized, three RHs–low RH (25±5 %, LRH), medium RH (45±5%, MRH), and high RH (85±5%, HRH)–were considered by adding dry or humid air into the dilution dryer. The size distribution function of infectious viruses based on the results of the plaque assay method was calculated following Eq. (4-1).

$$PFU/cm^3 = \frac{C_{PFU} \times V}{C_{Eff} \times Q_{inlet} \times \Delta \log d_p \times t} \quad (4\text{-}1)$$

where C_{PFU} is the virus concentration in the collection medium of the BioSampler, V is the volume of the collection medium of the BioSampler, C_{Eff} is the correction factor for the collection efficiency of the BioSampler for specific particle size, which is adopted from Hogan et al. (2005) and is listed in Table 4-1, Q_{inlet} is the inlet flow rate of DMA, t is the collection time of the BioSampler, and $\Delta \log d_p$ is the interval of specific particle size range set by the DMA.

Three different types of virus suspensions were tested. They were prepared by spiking 0.5 mL of virus stock in 50 mL of filtered sterile DI water, 0.25 % tryptone in filtered sterile DI water, and artificial saliva. Tryptone is derived from casein by enzymatic treatment that provides a source of peptides and amino acids for growing bacteria. When MS2 was aerosolized with tryptone, the stability of airborne MS2 over a wide range of RH was reported (Dubovi and Akers 1970). To preserve the infectivity of MS2 aerosols and simulate substances in the air that can contribute to encasement of viruses, the virus suspension was aerosolized with tryptone. The artificial saliva was used to emulate the situation where human beings are the source of viral aerosols. Components of the artificial saliva were taken from prior studies (Veerman et al. 1996; Wong and Sissions 2001; Aps and Martens 2005) and are listed in Table 4-2. Regarding the

protein components in the artificial saliva, mucin (one of major components of saliva) was added to the total protein concentration level in saliva. The mucin-containing saliva is the best substitute of natural saliva in rheological properties, and viscosity and elasticity of this medium are responsible for the protective role of saliva against desiccation (Vissink et al. 1984). Depending on the virus suspension in the nebulizer, the size of dry aerosols or droplet nuclei (d_p) can be calculated from the droplet diameter, d_d, according to Eq. (4-2) (Hinds 1999c).

$$d_p = d_d (F_v)^{1/3} \quad (4\text{-}2)$$

where F_v is the volume fraction of solid content in nebulizer suspension. The volume fraction of solid content for MS2 suspension in DI water, 0.25% tryptone solution, and artificial saliva was 9.9×10^{-4}, 3.5×10^{-3}, and 2.1×10^{-2}, respectively. After complete evaporation, the particle size of MS2 aerosols generated from DI water, tryptone solution, and artificial saliva was 0.10 d_d, 0.15 d_d, and 0.28 d_d, respectively.

Seven particle sizes were selected including (1) 30 nm, which is close to the nominal MS2 primary particle size, (2) 230 nm, which is the upper limit of particle size measured by the SMPS when the sample flow of the electrostatic classifier is set at 1.5 Lpm, and (3) 60 nm, 90 nm, 120 nm, 150 nm, and 200 nm, which provides information of intermediate sizes.

PCR assay

Before submission to PCR analysis, 4–mL samples were concentrated to 280 µL by using an Amicon ultracentrifugal device (UFC 810096, Millipore, Bedford, MA, USA) followed by RNA extraction with QIAamp Viral RNA mini kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions and stored at -80 °C. A previous study (O'Connell et al. 2006) of real-time fluorogenic reverse transcriptase (RT-PCR) assays for detection of MS2 was followed for design of primer and probe sequences. The GenBank accession number was

NC_001417. The sequences set for RNA replicase β chain were selected for testing because it is a critical component in infection process and more relevant than other genes. For reverse transcription (RT), 10 µL of reaction mixture prepared from 2 µL of 10X RT buffer, 0.8 µL of dNTPs, 2 µL of reverse primer, 1 µL of reverse transcriptase, and 4.2 µL of DNase/RNase free water was mixed with 10 µL of extracted viral RNA for a total final volume of 20 µL. The first RT step was carried out at 65 °C for 5 min and immediately quenched on ice for at least 1 min. The thermal cycling setting for RT was 10 min at 25 °C, 2 hrs at 37 °C, and 30 secs at 85 °C. The RT products (cDNA) were immediately cooled to 4 °C.

For RT-PCR, 5 µL of cDNA was added to 10 µL of TaqMan Universal Master Mix (Applied Biosystems), 1.25 µL of each primer (forward and reverse) and probe, and 5 µL of DNase/RNase free water to a final volume of 25 µL. All primers and probes were synthesized by Applied Biosystems. PCR was performed at 50 °C for 2 min, then at 95 °C for 10 min, followed by 50 cycles of 15 s at 95 °C and 60 s at 60 °C, on 7900HT fast real-time PCR system (Applied Biosystems). DNase/RNase free water was substituted for RNA to prepare the negative control.

Results and Discussion

MS2 aerosols generated with sterile DI water

Figure 4-2 shows the number-based and mass-based PSDs for aerosols generated from MS2 suspension in sterile DI water at LRH. The size distribution function of infectious count obtained by Eq. (4-1) is also presented. Similar trends of following mass-based PSD are also observed at MRH and HRH, which are presented in Appendix D (raw data are available in Appendix E). The PSD of aerosols monitored by the SMPS showed negligible variation during experiment indicating constant generation of PSD. Infectivity of MS2 in the nebulizer

suspension was analyzed before and after each experiment. Insignificant change in the infectivity proves negligible mechanical stress induced by the aerosolization process on the MS2 infectivity.

The plaque assay results were compared with the PSD of aerosols to investigate the number of infectious viruses as a function of particle size. Since sterile DI water having 0 PFU/mL (baseline) also generates aerosols, at very low concentration, the number of aerosol particles was corrected by subtracting the baseline PSD. Figure 4-3 shows the number of PFU per particle (N_{PFU}) as a function of particle size ranging from 30 to 230 nm at three RHs. Since the collection efficiency of the BioSampler depends on the particle size, N_{PFU} was corrected for that given particle size using the collection factor listed in Table 4-1. The theoretical N_{PFU} ($N_{Theo\ PFU}$) was calculated from the volume fraction of infectious MS2 in the solid content of the spray medium for the given particle size, according to Eq. (4-3).

$$N_{Theo\ PFU} = \frac{V_{dp} \times F_{MS2}}{\left(\frac{\pi}{6} d_{MS2}{}^{3}\right)} \tag{4-3}$$

where V_{dp} is the volume of the droplet nuclei, F_{MS2} is the volume fraction of infectious MS2 in the solid content of the spray medium obtained from the plaque assay for MS2 stock suspension (volume of infectious MS2/volume of freeze-dried MS2 stock), and d_{MS2} is the nominal size of MS2 (27.5 nm). As shown in Figure 4-3, more PFU was enumerated at all particle sizes at LRH compared to other RHs. Possible reasons for this dependence on RH include stability of MS2 aerosols at LRH and/or enumeration of more viruses at LRH than other RHs when the same particle size is compared. After wet dissemination, particle size changes due to evaporation, the rate of which depends on the surrounding RH. Therefore, for any same registered particle size set by the classifier at three RHs, there are more MS2 virions at LRH than at MRH and HRH due

to different evaporation rate. At the same RH, N_{PFU} increased as particle size increased with similar trend to $N_{Theo\ PFU}$, implying that the increase of N_{PFU} follows the increase of particle volume. This observation can be verified by conducting regression analysis of N_{PFU} as the dependent variable and particle size (d^n) as the explanatory variable. The n value at three RHs, which is presented as the slope of least squares regression line ($\ln(N_{PFU})$ vs. $\ln(d_p)$) in Table 4-3, can be compared to support this observation. As shown, the n value of N_{PFU} was in the vicinity of 3 at all three RHs, implying that the increase of N_{PFU} followed the increase of particle volume. Hogan et al. (2005) examined 25–, 120– and 300–nm MS2 aerosols and their results indicated increasing N_{PFU} as particle size increased, although they did not report the particle volume relationship.

Total viruses, including infectious and non-infectious viruses, of a given particle size were investigated by quantifying RNA in the aqueous collection medium after considering the correction factor for collection efficiency of the BioSampler. The threshold cycle (C_T) value of the sample from RT-PCR was compared with a standard curve obtained by plotting C_T value for serial dilutions of commercially available MS2 RNA (Roche Diagnostics, Indianapolis, IN, USA) against the experimental RNA amount. Using 1.0×10^6 g/mol as the molecular weight of MS2 RNA (Kuzmanovic et al. 2003), the number of MS2 RNA in the samples was calculated with the conservative assumption that one MS2 RNA represents one MS2 virion. The number of MS2 RNA per particle (N_{RNA}) was then determined by dividing it by the total aerosol particles measured by the CPC following Eq. (4-4):

$$N_{RNA} = \frac{RNA(ng)\,in\ the\ Sample \times (10^{-9}\,g/ng)}{\left(\frac{1.0\times10^6\,g}{1mol}\right)\times\left(\frac{1mol}{6.02\times10^{23}\,molecules}\right)\times number\ of\ aerosol\ particles\times C_{Eff}} \qquad (4\text{-}4)$$

It should be noted that N_{RNA} includes any fragment of MS2 RNA containing the target sequence for PCR as well as infectious RNA. Biodegradation of RNA having target sequence can lead to underestimated PCR results; special care of storage and sample handling is needed to prevent underestimates.

PCR analysis was conducted for two experimental sets and for selected particle sizes. Table 4-4 shows N_{RNA} for several particle sizes at three RHs as well as the theoretical value of N_{RNA}. Similar to $N_{Theo\ PFU}$, theoretical N_{RNA} ($N_{Theo\ RNA}$) was also calculated using Eq. (4-3) except that total MS2 instead of infectious MS2 was considered. In the equation, F_{MS2} was calculated for total MS2 with the assumption that the freeze-dried MS2 stock is mainly composed of MS2 particles with negligible impurity. As shown in Table 4-4, the enumeration of MS2 RNA in a given particle size increased as RH decreased due to more contribution of solid content to the particle size. At the same RH, the value generally increased as particle size increased. The increased rate of N_{RNA} at three RHs was generally much less than that of $N_{Theo\ RNA}$, which can be confirmed by the same regression analysis applied for comparison of N_{PFU} at three RHs. Table 4-5 lists the n value (the slope of the regression analysis) at three RHs. As shown, the presence of total viruses (N_{RNA}) in aerosol particles increased in proportion to particle surface area (n = 2) or an even lower dimension. The difference between experimental N_{RNA} and $N_{Theo\ RNA}$ can be attributed to the discontinuous distribution or generation of MS2 particles in the suspension. Whereas $N_{Theo\ RNA}$ was calculated with the assumption that MS2 particles are uniformly dispersed, in reality the distribution of MS2 particles in the nebulizer suspension is not uniform. The presence of MS2 aggregates in the nebulizer suspension caused by hydrophobic interactions between neighboring protein capsids has been observed in previous studies (Hogan et al. 2004; Balazy et al. 2006). Therefore, MS2 virions can be present in aerosols as individuals, as

aggregates or attached to the surface of the solid content. At the same time there are particles that contain no MS2.

The stability of MS2 aerosols was investigated by comparing N_{PFU}/N_{RNA} (*i.e.*, infectious MS2/total MS2) of select particle sizes at three RHs as shown in Table 4-6. N_{PFU} in a unit RNA was significantly higher at LRH than at MRH and HRH (one-way ANOVA, p-value < 0.05), indicating stability and preservation of MS2 infectivity in aerosols at LRH. No significant difference was observed between the value at MRH and HRH (unpaired Student's t-test, p-value > 0.05), indicating similar survival capacity. This observation can be attributed to the increase in air– to–water interface at increased RHs, which results in the exposure of aerosols to unbalanced force leading to a decrease of N_{PFU} (Adams 1948). N_{PFU}/N_{RNA} generally increased as particle size increased at MRH and HRH in spite of the adverse effect at increased RHs. This result demonstrates the shielding effect of bigger particles. Indeed, MS2 aerosols, which are less stable at 50% and 85% than at 25% RH, can be protected by forming aggregates to reduce exposure to the adverse influence of increased RH. Meanwhile, at LRH, N_{PFU} in a unit RNA differed insignificantly among the various particle sizes investigated. This shows that, without the adverse effect of RH, shielding due to aggregation decreases in importance to survival.

MS2 aerosols generated from tryptone solution

Experiments were also conducted with tyrptone solution as the aerosolization medium. As shown in Figure 4-4 for LRH, the presence of tryptone in the nebulizer suspension shifted the PSD towards the bigger particle size range compared to the MS2 aerosols generated from sterile DI water as Eq. (4-2) predicts. The PSD of infectious viruses was between number- and mass-based PSD, *i.e.* its n value was between 2 and 3 as shown in Table 4-3. At other RHs, a similar phenomenon was also observed (Appendix D). The N_{PFU} of a given particle size was also

calculated by following the same equation used for sterile DI water (shown in Figure 4-5). Similar to the MS2 aerosols generated with sterile DI water (shown in Figure 4-3), N_{PFU} increased as particle size increased. However, the values at three RHs increased less with increasing particle size than $N_{Theo\ PFU}$ (n = 3), and were also lower than those for sterile DI water (Table 4-3). It is plausible that the abundance of tryptophan in tryptone induces hydrophobic interaction with MS2 protein and also provides surface for MS2 to reside on or attach to. This phenomenon can cause N_{PFU} increase in proportion to surface rather than to volume.

It should also be noted that N_{PFU} was significantly lower than that generated from sterile DI water at LRH. The reason for this phenomenon is the contribution of tryptone to the solid content of droplet nuclei, which leaves less room for MS2. This contribution can be verified by analyzing N_{RNA} in the samples. By using the calculation used for MS2 aerosols generated from sterile DI water, N_{RNA} of select particle sizes at three RHs was calculated (shown in Table 4-7). Clearly, N_{RNA} was significantly smaller than that of MS2 aerosols generated from DI water (Table 4-4), due to the significant solid fraction resulting from the presence of tryptone. The N_{RNA} of a given particle size was higher at LRH than at MRH and HRH due to increased solid contents; meanwhile, insignificant difference was observed between MRH and HRH.

Table 4-8 presents N_{PFU}/N_{RNA} at three RHs. At LRH, N_{PFU}/N_{RNA} shows similar values among different particle sizes. The result demonstrates that when viruses are not exposed to the adverse effect of increased RH, the presence of tryptone exerts no protective effect. N_{PFU}/N_{RNA} at HRH was significantly higher than that at LRH and MRH, as well as at HRH for MS2 aerosols generated from sterile DI water. This observation can be explained by the encasement effect of tryptone for MS2 aerosols in the hostile condition of HRH. A similar study demonstrated high recovery of MS2 aerosols at all RHs ranging from 20 to 80% due to the protective effect of

tryptone (Dubovi and Akers 1970). Within the same context, increased N_{PFU}/N_{RNA} for MS2 aerosols generated from tryptone solution than that for MS2 aerosols generated from suspensions in DI water was expected at MRH. However, as seen in Tables 4-6 and 4-8, insignificant increase was observed. This observation, along with the significant decrease of N_{PFU} compared to that for sterile DI water at LRH, suggests an adverse effect of tryptone at LRH and MRH, rather than a protective effect. This result can be attributed to the supersaturated condition of tryptone in droplet at LRH and MRH. Although this observation does not agree with the Dubovi and Akers (1970) study in the aspect that they observed high recovery of MS2 at LRH and MRH, Trouwborst and de Jong (1973) demonstrated that phenylalanine does not exert a protective effect for MS2 aerosols under supersaturated conditions. They mentioned that crystals or the process of crystallization can be deleterious to MS2 aerosols. As the RH keeps decreasing, droplets may reach the crystallization RH (CRH), which is the maximum RH at which solutes maintain the aqueous phase without experiencing crystallization at a supersaturated condition. The CRH is always below the deliquescence RH (DRH). It was reported that the DRH and CRH of ammonium sulfate are 80% and 40%, respectively (Seinfeld and Pandis 1998). Also, some common components of ambient aerosols have a DRH between 70% and 85%. Although the CRH of these components was not reported, it is reasonable to expect that the value is similar to that of ammonium sulfate unless the species are not hygroscopic. Within the same context, the CRH of various components of the spray medium can be around 40%, which is about the MRH investigated in this study.

MS2 aerosols generated with artificial saliva

Figure 4-6 shows the PSD of number-based, mass-based, and infectious counts as a function of particle size at LRH. Apparently, the PSD was shifted to an even bigger particle size

range compared to the PSD generated from tryptone solution due to the increased volume fraction of solid materials in the nebulizer suspension. The PSD of infectious viruses followed a lower order dependence on dimension, between number and area distributions, as shown in Table 4-3. The results at other RHs show a similar pattern and are presented in Appendix D.

Figure 4-7 shows N_{PFU} as a function of particle size at three RHs. Compared to the MS2 aerosols generated from tryptone solution and sterile DI water, there was less increment as particle size increased at three RHs. There are two possible reasons for this phenomenon: (1) negligible shielding effect of bigger particles due to insufficient amount of MS2 virus to be aggregated, and (2) adverse effect of saliva components on viral aerosols. In terms of the amount of MS2 viruses, the N_{RNA} values for MS2 generated from artificial saliva are similar to those for tryptone soluton, as shown in Tables 4-7 and 4-9. Since the latter presented a shielding effect, MS2 aerosols generated from artificial saliva should have sufficient N_{RNA} to present a shielding effect of aggregates. The fact that N_{PFU} is low implies that MS2 virions in aerosols do not aggregate well to achieve a shielding effect. To address this issue, one should recall that the artificial saliva used in this study is a mucin-containing medium. Mucin has an oligosaccharide chain containing numerous hydrophobic regions, which are responsible for its sticky property (Mehrotra et al. 1998; Zalewska et al. 2000). In a later study (Habte et al. 2006), it was observed that mucin aggregates HIV-1 (human immunodeficiency virus type 1) leading to an enhanced filtration through 0.45–μm pore size cellulose acetate filters. Therefore, it can be inferred that mucin induces hydrophobic interaction with MS2 protein, thus reducing MS2 aggregation by itself. The lack of shielding effect of aggregates is verified by the lower slope value shown in Table 4-3.

The negligible increase of N_{PFU} as particle size increases can also result from the adverse effect of saliva components. The adverse effect of saliva on the stability of viral aerosols has been reported in a previous study (Barlow and Donaldson 1973). They observed that foot-and-mouth disease viral aerosols were more unstable when generated from bovine salivary fluid than from cell culture fluid at HRH, and they postulated the presence of an "inactivating factor" in the saliva as the reason for instability of viral aerosols. In later studies (Fox et al. 1988; Bergey et al. 1994; van der Strate et al. 2001; Hartshorn et al. 2006), an antiviral effect of saliva on HIV-1 and influenza A virus was observed and some proteins of saliva such as lactoferrine, agglutinin, and mucins were proven to be the inactivating factors.

Table 4-10 shows N_{PFU}/N_{RNA} at three RHs. The values at LRH and MRH were similar to those for MS2 aerosols generated from tryptone solution, indicating a similar adverse effect. At HRH, the values were lower than those from the tryptone medium. Both inactivation effects from salivary components and from the air/water interface can be factors. The protective effect of tryptone at HRH was not observed for artificial saliva, showing again the adverse effect of saliva components. However, no synergistic effect of these two factors was observed since the N_{PFU}/N_{RNA} values were similar to those for MS2 aerosols generated with DI water, which were adversely influenced only by the air/water interface.

Distribution of MS2 in aerosol particles generated from different spray media

As presented in Tables 4-3 and 4-5, the distribution of MS2 including both infectious and total (infectious and non-infectious) viruses along the aerosol size ranging from 30 to 230 nm was investigated. The *n* values for N_{RNA} and N_{PFU} for aerosols generated from DI water and from tryptone solution were different, although the difference was less for tryptone solution than for sterile DI water. On the other hand, MS2 aerosols generated from saliva showed a much

smaller n value for both N_{PFU} and N_{RNA}. Since N_{PFU} represents only infectious viruses while N_{RNA} includes fragments of nucleic acid and non-infectious viruses, and infectious viruses, these two values can be quite different in the presence of other substances.

To assess the influence of spray media on N_{PFU} and N_{RNA}, two-way ANOVA analysis was conducted. For N_{RNA}, the n value showed negligible difference among the media (p-value > 0.05). Meanwhile, the n value of N_{PFU} exhibited a significantly different increase rate (p-value < 0.05); it decreased as solid material in the spray medium increased in the order DI water (2.9), tryptone solution (2.4), and artificial saliva (1.1). Note that the values presented in parenthesis are averaged at three RHs. This difference between N_{RNA} and N_{PFU}, and also among spray medium for N_{PFU} can be attributed to a combination of several factors, including shielding and encasement effects. The infectious viruses (N_{PFU}) protected by shielding or encasement effects increase generally in proportion to volume distribution as particle size increases. Regarding MS2 aerosols generated from artificial saliva, the adverse effect of saliva and negligible shielding effect contribute to the similar results between N_{RNA} and N_{PFU} and to a much smaller n value than the other spray media.

The PSD of infectious viruses (PFU/cm^3, shown in Figures 4-2, 4-4 and 4-6) for different spray media showed that infectious viruses are more abundant from a relatively pure virus suspension (sterile DI water) than from solid-containing spray media (tryptone solution and artificial saliva) at LRH. It should be emphasized that the size range for this observation is from 30 nm to 230 nm. If the window were expanded to include bigger particle sizes, it is possible that more MS2 from a solid-containing spray medium would be enumerated than that from a relatively pure virus suspension. This phenomenon is supported by the theory of aerosol nebulization (Eq. 4-2). As shown, the aerosol diameter is determined by the droplet diameter

and volume fraction of solid materials in the spray medium. Figure 4-8 illustrates the theoretical shrinkage of droplets to droplet nuclei for MS2 aerosols generated from these three different nebulizer suspensions. The droplet nuclei resulting from the same droplet get smaller as the solid fraction in the nebulizer suspension decreases. For instance, a droplet of 2000 nm shrinks to a nucleus about 200 nm from sterile DI water, while the corresponding droplet nuclei from tryptone solution and from artificial saliva are about 300 nm and 560 nm, respectively. Since the amount of MS2 stock suspension in the nebulizer is the same for all three spray media, the total aerosols of MS2 aerosols generated from the nebulizer should also be the same. Therefore, it is reasonable to expect that aerosols of 300 nm and 560 nm generated from solid-containing spray media contain similar amounts of N_{RNA} and N_{PFU} to that observed for 200–nm aerosols generated from DI water.

DISCUSSION

Regarding the effect of spray medium, two fundamental questions arise: (1) does adding tryptone really help preservation of MS2 aerosols or disseminate MS2 aerosols more effectively than from DI water? and (2) does saliva help reduce the hazard of viral aerosols? The latter question is of particular interest in relation to recent human cases of influenza A (H1N1) virus infection and its rapidly evolving situation.

The presence of tryptone in the spray medium results in two contrary phenomena. In a dry environment, tryptone can be deleterious to MS2 due to crystallization under supersaturated conditions in aerosols. Meanwhile, sensitive MS2 at increased RHs can be protected by the encasement effect of tryptone, and thus enumerated in a relatively larger numbers. This statement can be verified by comparing the results with those from sterile DI water. Although the number of total viruses (N_{RNA}) in aerosols decreased due to the contribution of tryptone to the

aerosol size, the number of infectious viruses (N_{PFU}) at HRH was similar to that from sterile DI water. In other words, at the optimal condition for the stability of viral aerosols (LRH), the most effective way to disseminate viral aerosols is to use a pure virus suspension. On the other hand, the presence of a protective material in the spray medium is a key factor for the spread of viral aerosols at sensitive conditions.

In addition to the stability of viral aerosols and the presence of protective materials, the effectiveness of spreading viruses depends on the aerosol size. It was already addressed earlier that the size of droplet nuclei is affected by the solid fraction in the spray medium and thus the PSD of aerosols generated from different spray media will be present in different size ranges. Depending on the particle size considered, the effective virus suspension for disseminating viruses varies. If a bigger particle size is desired, a solid-containing virus suspension will be a more effective way to spread viruses than a relatively pure virus suspension. This study proves that both environmental factors (*e.g.,* RH) and substances in the virus suspension play a significant role in the fate of viral aerosols. Furthermore, these factors can be protective or deleterious, depending on the combination.

For MS2 aerosols generated from artificial saliva, the adverse effect of salivary protein was observed. Although certain viruses including adenovirus and vaccinia virus, are not or little affected by salivary proteins (Bergey et al. 1993; Malamud et al. 1993), the antimicrobial role of saliva has been extensively observed. Hence, in the scenario that human beings are the source of viral aerosols, the consequence of spread of viral aerosols can be less profound than expected because of the resulting lower number of infectious viruses. As discussed earlier, the presence of solid materials (saliva components) can reduce the amount of virions or lower the degree of aggregation in aerosols compared to the pure virus suspension. From these observations for

tryptone and saliva, it can be informed that both concentration and nature of solid materials dissolved in the spray medium determine the size and fate of viral aerosols at any given RH.

As discussed previously, three concerns relating to specific characteristics of viruses are small particle size, shielding effect, and encasement effect of substances. We observed both a shielding effect of aggregates and an encasement effect by the presence of inert materials for 200−nm viral aerosols. It should be emphasized that aerosols of this size are small enough to reach the alveolar region of the lungs, and inhalation of one such single particle can easily attain the minimum infectious dose of virus with enhanced shielding and the encasement effect. For example, the N_{PFU} resulting from the penetration of a single 200−nm particle through a filter or respirator is equivalent to the N_{PFU} resulting from the penetration of 100 30−nm particles of MS2 generated from DI water at HRH.

Although the current study characterized one specific species (MS2 bacteriophage), general characteristics applicable to other viral aerosols can be deduced from our findings. The shielding effect of small aggregates is a common characteristic of general viruses because of their tiny primary particle size and aggregated airborne state. In addition, as observed in the encasement effect of tryptone, inert materials (*e.g.*, dust in air or substances generated with viruses) can exert a protective influence on viral aerosols in adverse conditions. These two general properties can contribute to the survival of viruses in otherwise hostile circumstances (*e.g.*, sensitive RH and temperature) or even inactivation treatments, and to subsequent initiation of infectivity and transmission of disease.

Table 4-1. Collection efficiency of the BioSampler for select particle sizes adopted from Hogan et al. (2005)

Particle diameter (nm)	Collection efficiency (%)
30	14
60	8
90	5
120	4
150	4
200	4
230	5.4

Table 4-2. Components of artificial saliva

Components	Content	Components	Content
$MgCl_2 \cdot 7\ H_2O$	0.04422 g	$(NH_2)_2CO$	0.1212 g
$CaCl_2 \cdot H_2O$	0.1288 g	NaCl	0.876 g
$NaHCO_3$	0.42 g	KCl	1.0416 g
0.2 M KH_2PO_4	7.7 mL	Mucin	3 g
0.2 M K_2HPO_4	12.3 mL	Tissue culture medium (DMEM)	1 mL
NH_4Cl	0.108 g	Water	979 mL
KSCN	0.194 g	pH	7

Table 4-3. Slope of least squares regression line for N_{PFU} vs. particle size for different MS2 suspensions at three relative humidities

MS2 suspension in Nebulizer	Slope of least squares regression line (R^2)		
	Low RH	Medium RH	High RH
DI water	2.8 (0.8)	3.0 (0.9)	2.9 (0.8)
Tryptone solution	2.4 (0.9)	2.9 (0.9)	2.1 (0.8)
Artificial saliva	1.4 (0.8)	1.4 (0.9)	0.6 (0.8)

Table 4-4. N_{RNA} for MS2 aerosols generated from sterile DI water at three relative humidities

Particle diameter (nm)	N_{RNA}						$N_{Theo\ RNA}$
	Low RH		Medium RH		High RH		
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2	
30	0.97	0.94	0.66	0.93	N/A	0.05	1.3
60	3.22	N/A*	2.20	N/A	4.04	N/A	10
90	9.72	1.62	N/A	0.38	4.08	0.99	35
120	16.32	8.09	12.17	0.49	9.63	0.64	82
150	39.50	N/A	18.25	N/A	12.52	N/A	160
200	51.17	20.69	31.86	14.11	12.11	2.67	380
230	64.96	N/A	52.26	N/A	3.56	N/A	580

* Not available

Table 4-5. Slope of least squares regression line for N_{RNA} vs. particle size for different MS2 suspensions at three relative humidities.

MS2 suspension in Nebulizer	Slope of least squares regression line (R^2)					
	Low RH		Medium RH		High RH	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
DI water	2.1 (0.9)	1.6 (0.8)	2.1 (0.9)	1.0 (0.2)	1.1 (0.8)	2.0 (0.9)
Tryptone solution	1.8 (0.9)	1.9 (0.9)	1.4 (0.6)	1.5 (0.8)	2.9 (0.9)	1.3 (0.7)
Artificial saliva	1.4 (0.9)	1.5 (0.9)	0.9 (0.9)	1.3 (0.8)	1.1 (0.8)	1.0 (0.8)

Table 4-6. N_{PFU} / N_{RNA} for MS2 aerosols generated from sterile DI water at three relative humidities

Particle diameter (nm)	Log (N_{PFU}/N_{RNA})					
	Low RH		Medium RH		High RH	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
30	-5.1	-5.4	-7.5	-6.6	N/A	-6.5
90	N/A	N/A	N/A	N/A	-6.6	-6.2
120	-5.5	-5.7	-6.9	-5.7	-6.4	-6.1
200	-5.2	-4.4	-5.7	-6.1	-6.0	-5.9

Table 4-7. N_{RNA} for MS2 aerosols generated from tryptone solution at three relative humidities

Particle dia. (nm)	N_{RNA}						$N_{Theo\ RNA}$
	Low RH		Medium RH		High RH		
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2	
30	0.18	0.58	0.07	0.26	N/A	0.45	0.38
60	0.39	N/A	0.19	N/A	N/A	N/A	3.1
90	0.60	N/A	0.39	N/A	0.27	0.50	10
120	2.80	4.91	1.65	1.07	0.46	1.98	25
150	3.79	N/A	1.45	N/A	1.19	N/A	48
200	5.65	27.73	2.38	6.45	2.61	6.71	110
230	5.46	N/A	0.45	N/A	3.80	N/A	160

Table 4-8. N_{PFU} / N_{RNA} for MS2 aerosols generated from tryptone solution at three relative humidities

Particle dia. (nm)	Log (N_{PFU}/N_{RNA})					
	Low RH		Medium RH		High RH	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
30	-6.1	-6.2	-6.5	-6.5	N/A	-5.7
90	N/A	N/A	N/A	N/A	-4.7	-5.4
120	-6.8	-6.1	-6.3	-6.2	-4.3	-5.5
200	-5.7	-6.2	-5.4	-5.7	-4.9	-5.1

Table 4-9. N_{RNA} for MS2 aerosols generated from artificial saliva at three relative humidities

Particle diameter (nm)	N_{RNA}						$N_{Theo\ RNA}$
	Low RH		Medium RH		High RH		
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2	
30	0.3	0.3	0.4	0.4	0.4	0.6	0.05
120	2.3	2.4	1.1	1.3	1.0	1.3	3.8
200	5.0	6.2	2.5	6.1	4.3	5.2	17

Table 4-10. N_{PFU} / N_{RNA} for MS2 aerosols generated from artificial saliva at three relative humidities

Particle diameter (nm)	Log (N_{PFU}/N_{RNA})					
	Low RH		Medium RH		High RH	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
30	-6.6	-6.2	-6.3	-6.4	-5.7	-6.1
120	-6.9	-6.5	-5.8	-6.2	-5.9	-6.1
200	-6.4	-6.9	-6.0	-6.3	-6.4	-6.5

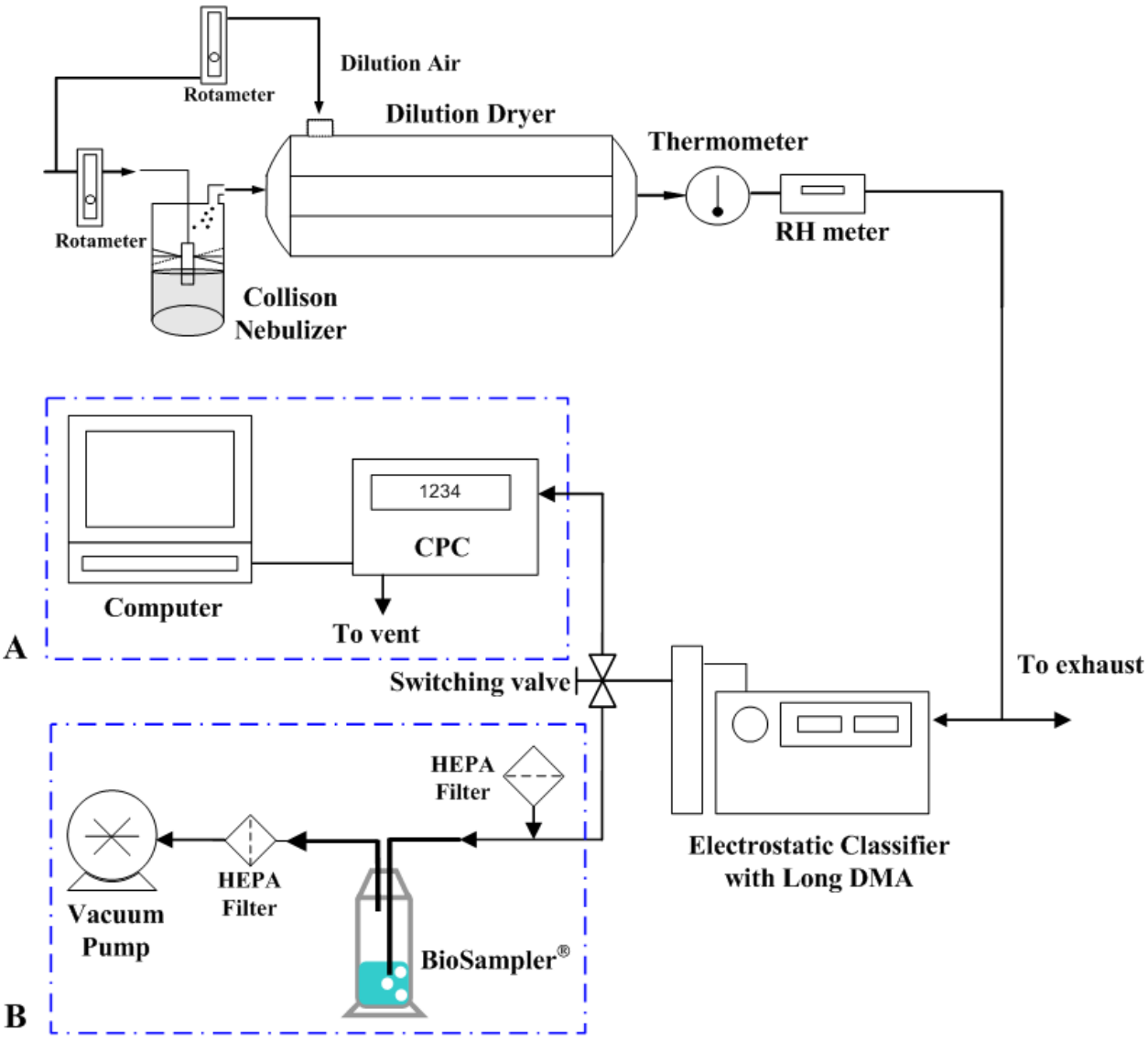


Figure 4-1. Conceptual schematic of the experimental set-up: A) Measurement of particle size distribution; B) Collection of viral aerosols of selected size.

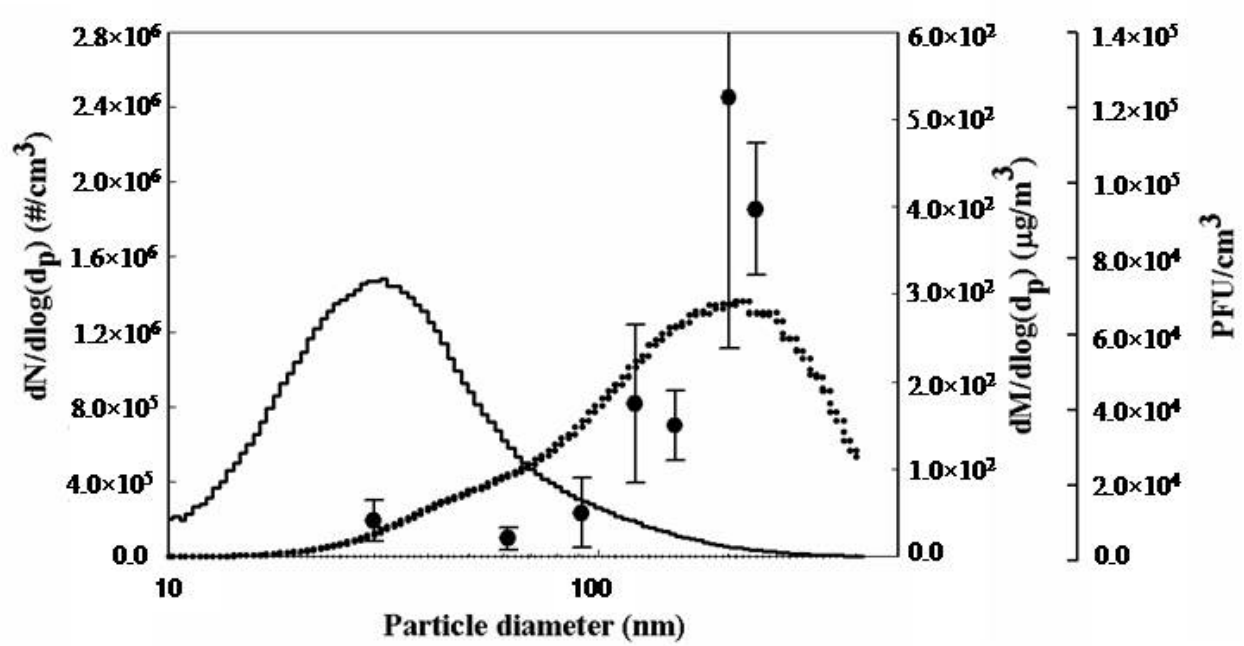


Figure 4-2. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from sterile DI water at low relative humidity.

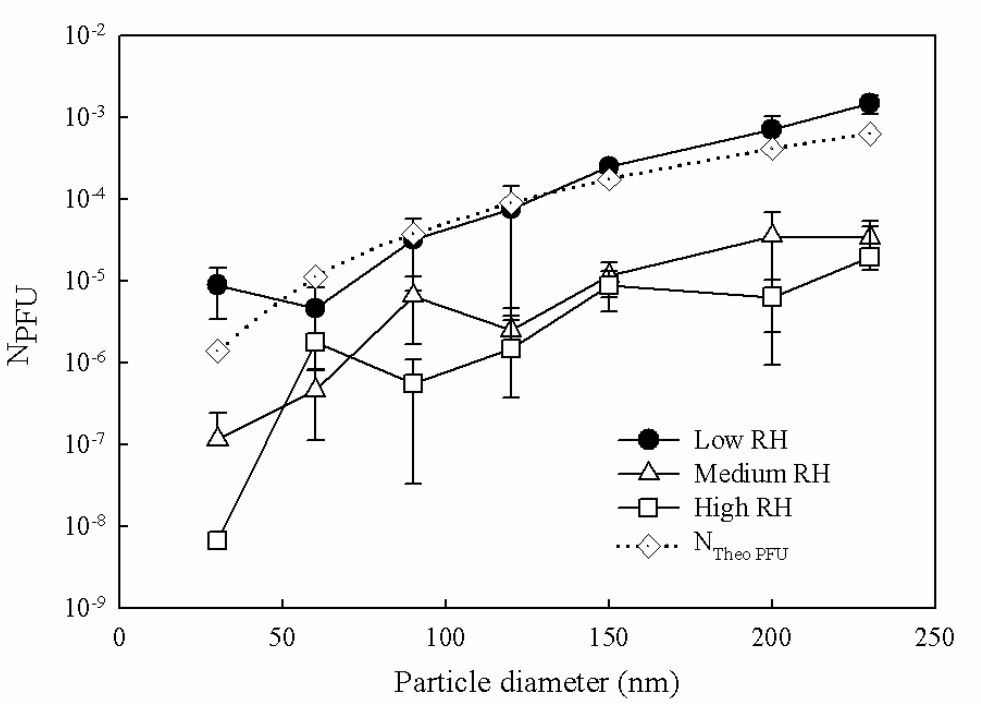


Figure 4-3. N_{PFU} for MS2 aerosols generated from sterile DI water at three relative humidities. Data shown are the mean of three repetitions with error bars representing standard error.

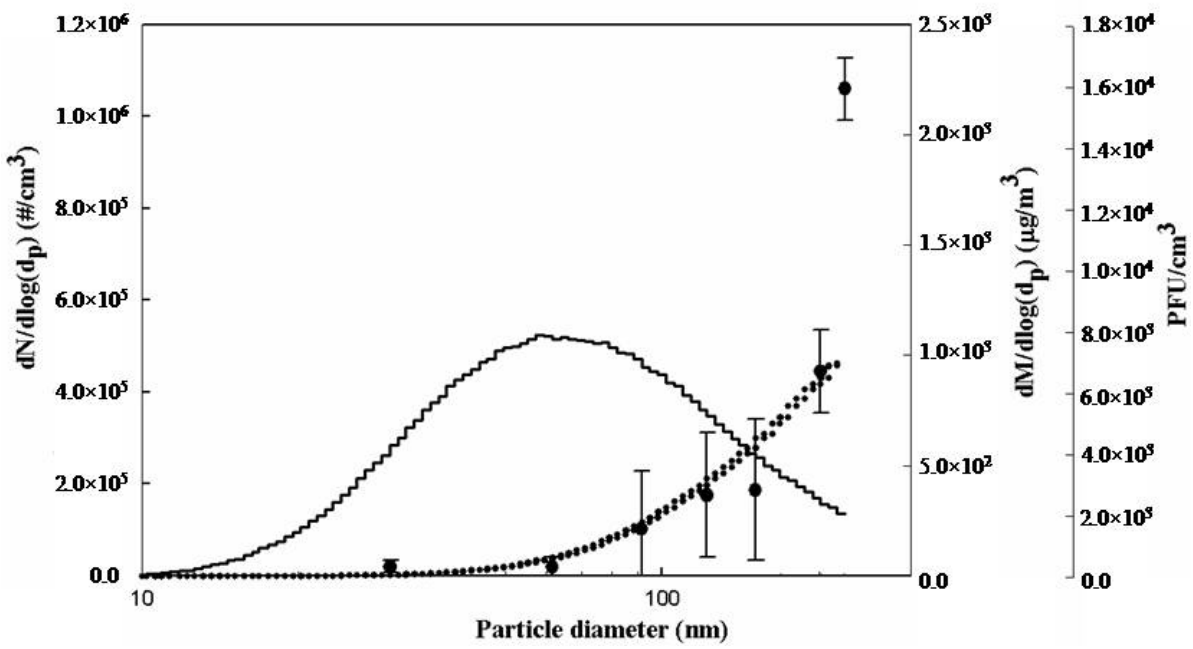


Figure 4-4. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from tryptone solution at low relative humidity.

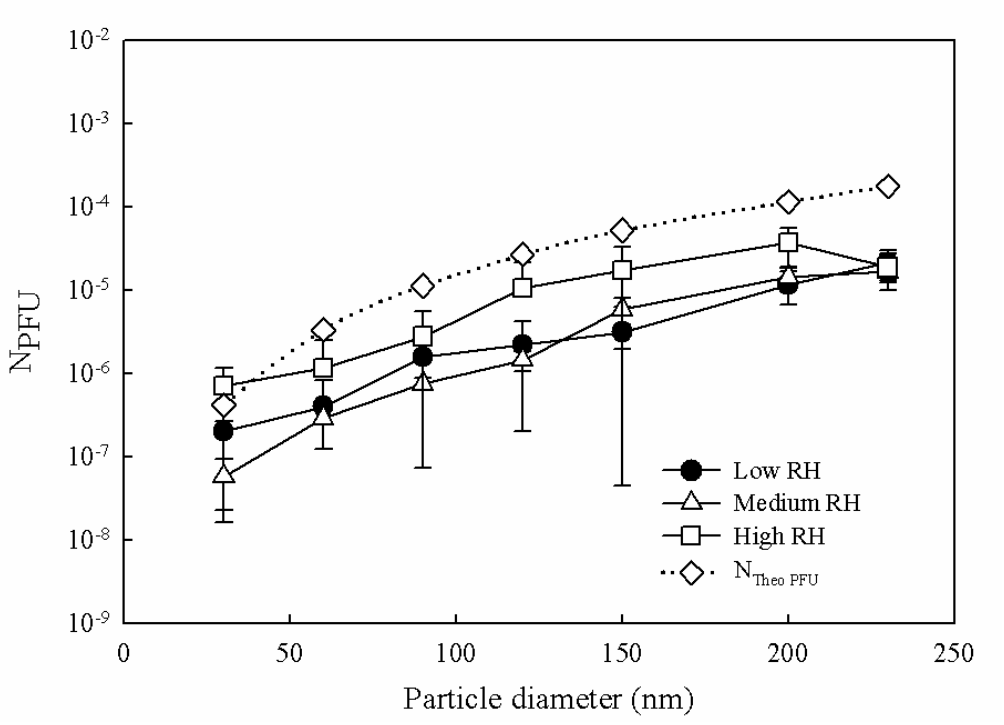


Figure 4-5. N_{PFU} for MS2 aerosols generated from tryptone solution at three relative humidities. Data shown are the mean of three repetitions with error bars representing standard error.

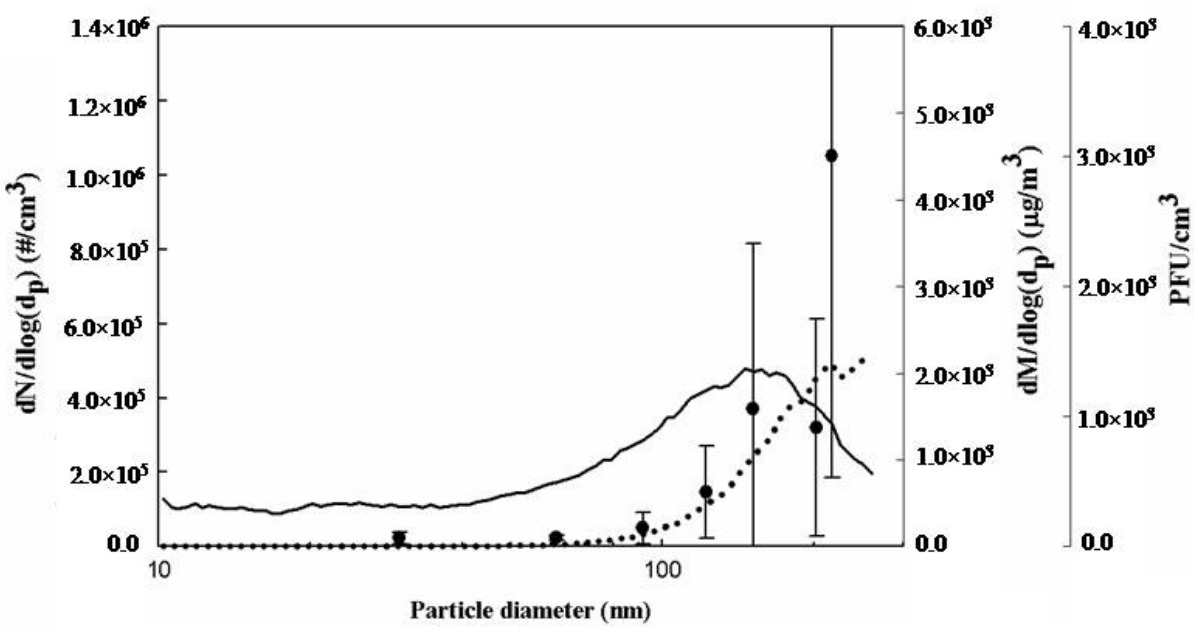


Figure 4-6. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from artificial saliva at low relative humidity.

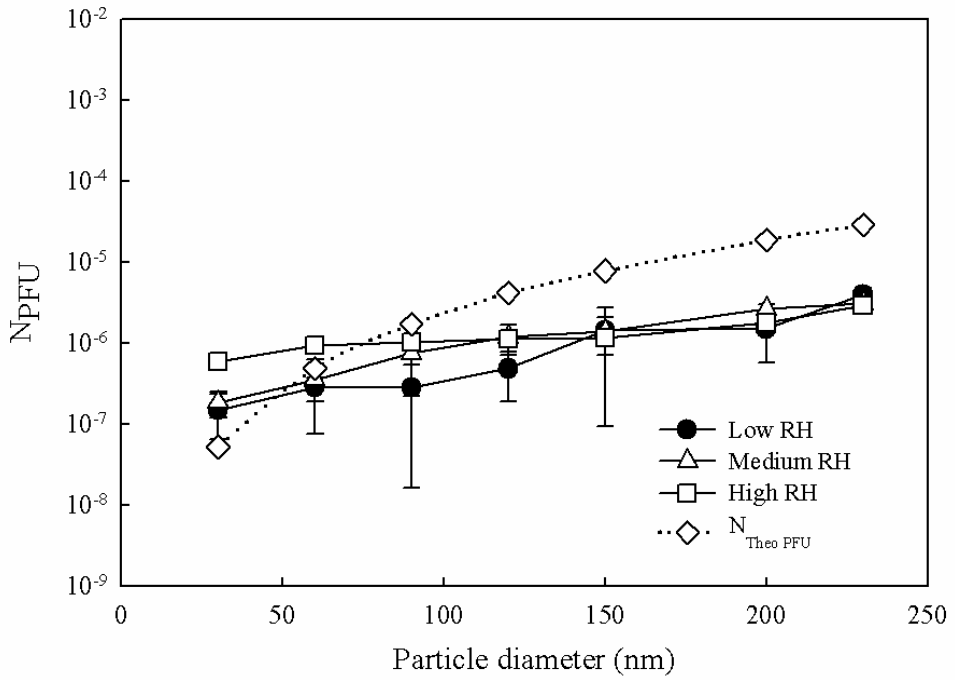


Figure 4-7. N_{PFU} for MS2 aerosols generated from artificial saliva at three relative humidities. Data shown are the mean of three repetitions with error bars representing standard error.

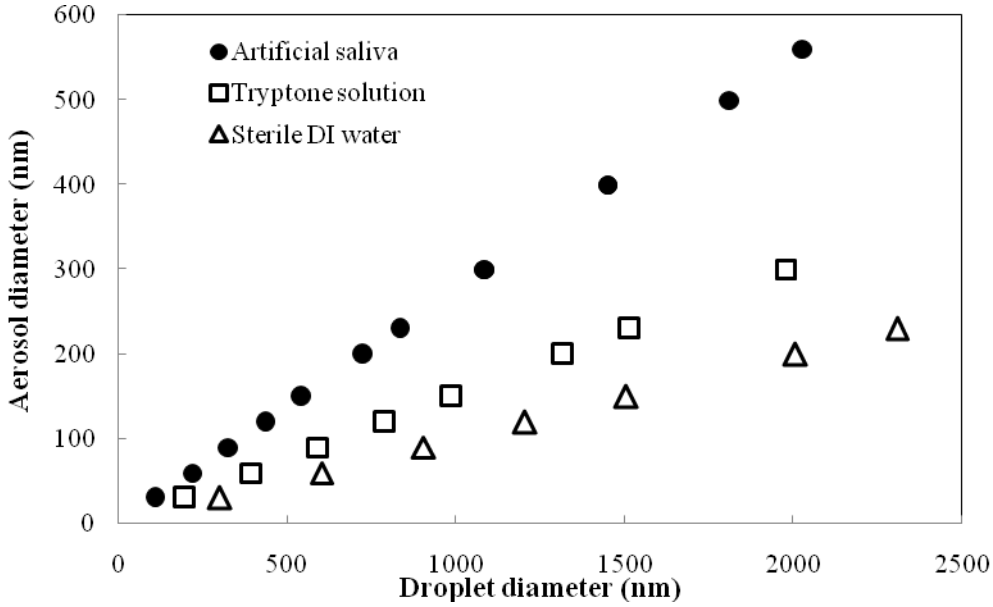


Figure 4-8. Theoretical droplet nuclei diameter as a function of droplet diameter for different nebulizer suspnesions at low relative humidity.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Both treated and untreated filters exhibited high viable removal efficiency (> 99.996%) for bacterial spores in various environmental conditions with negligible variation in pressure drop. This great performance of test filters did not deteriorate over the experimental duration (*i.e.*, 10 hr or 4 hr). Viability of spores collected on the filter was investigated by extracting them from the filter and presented as the survival fraction. A higher survival fraction on the untreated filter than that on the treated filter was reported at RT/LRH. However, the survival fraction of treated filters at RT/HRH and HT/HRH was similar to that of untreated filters tested at RT/LRH. Loss of iodine by sublimation and dissolution at HT and HRH was responsible for the indifference. As electret filter media, both treated and untreated filters presented high retention capability for negatively charged bacterial spores and thus reduced potential hazard from the release of spores from the media.

For the viral aerosol filtration experiment, new filter media different from those used in the bacterial spore experiment were used. The iodine-treated filter presented high removal efficiency for viral aerosols with low breathing resistance (significantly lower pressure drop than the NIOSH regulation) under various environmental conditions. Both treated and untreated filters presented similar extracted fractions, indicating insignificant difference in the infectivity of viruses on both filters. This observation can be attributed to the shielding effect of MS2 aggregates on the filter and/or high retention capability of the filter due to electrostatic interaction between charged filter media and viral particles making any difference indistinguishable. Meanwhile, it demonstrates again high retention capability that can minimize reaerosolization and prevent air filters to be a potential source of microbial contamination. As an inactivation mechanism of the iodine-treated filter, transfer of a lethal dose of iodine from the

filter to the MS2 aerosols during its flight through the filter, which was proposed previously, was verified. By comparing the experimental results of BSA and thiosulfate solution, we concluded that reaction of MS2 in the collection medium of the sampling device with iodine released from the filter was also occurring as a competing inactivation mechanism. This realization requires that these two inactivation pathways be factored in the design of the assessment methodology and interpretation of its results. After completing these studies we learned that the threshold iodine concentration for inactivation is low— ~ 0.5 ppm in water as disclosed by Triosyn Corp. (Messier 2009)—so the concentrations of I_2 at which we operated are large enough that the experimental results of the impinger with PBS were obscured by the free iodine in the collection medium of the impinger. Specifically, measured concentrations of released iodine collected with the bioaerosols in the impinger were sufficient to inactivate microbes collected in the impinger and to obscure the inactivation process of iodine on the microbes. In the present experimental configuration, it takes only a few milliseconds for microbes to penetrate the iodine-treated filter before being collected in the impinger, indicating a transient reaction time of microbes with iodine in the air phase. Therefore, even if microbes penetrating the iodine-treated filter accumulate iodine molecules, they can still be viable unless iodine in the surface of microbes work its way in. The experimental result of thiosulfate solution presenting survival of microbes penetrating the iodine-treated filter implies that inactivation process of iodine is not completed in such a short time and must be relatively slow on that time scale. In this context, one cannot exactly interpret the experimental result that BSA allows inactivation of half of penetrating MS2, but one can conclude that some transfer of loosely bound iodine to BSA from aerosols occurs, allowing insufficient reaction time for the labile iodine to inactivate the surviving fraction of microbes.

The infectious and total MS2 viruses as a function of aerosol size in the ultrafine and submicrometer size range, influenced by relative humidity and spray medium, were investigated with bioassay and PCR analysis, respectively. Both infectious and total viruses increased as particle size increased although the increase rate varied depending on several factors such as protective effect and nature of solid content in spray medium. With the stability at LRH and greater solid fraction, the number of infectious viruses was significantly higher at LRH compared to MRH and HRH for MS2 aerosols from DI water. The sensitivity of MS2 to increased RH can be attributed to the unbalanced force of air-water interface. In the presence of solid content in viral aerosols (tryptone and artificial saliva), the enumeration of MS2 in aerosols decreased due to greater contribution of solid contents to the aerosol size. The shielding effect of aggregates and encasement effect of tryptone resulted in enhanced MS2 infectivity than expected one by reducing contact of viral aerosols to the adverse factor such as environmental conditions. On the other hand, artificial saliva exerted adverse effect on the infectivity of viral aerosols and yielded negligible shielding effect. The present study demonstrates that even one single aerosol particle can have sufficient infectious virions to exceed the minimum infectious dose due to shielding and encasement effect. It is therefore critically important to develop new technologies that can more effectively protect the public from airborne viral pathogens.

Topics for future research in this area include investigation of the effect of the presence of foreign aerosols on the performance of the iodine-treated filter and on the inactivation process of iodine on microbes. The presence of foreign aerosols may hinder the exertion of biocidal effect by resulting in either masking of the iodine-treated site with these particles or parasitic consumption of oxidizing equivalents. Furthermore, these substances can serve as nutrients for the growth of collected microorganisms, eventually resulting in the inhalation of bioaerosols

from re-entrainment. Full evaluation of such a condition will determine its application to diverse scenarios. Clarification of inactivation mechanisms for airborne biological agents after transit through the iodine-treated filter should be investigated, to identify transport mechanisms and reaction pathways of iodine that operate on the time scale of my experiments.

APPENDIX A
RAW DATA OF BACTERIAL SPORE EXPERIMENTS

Table A-1. Impactor results at room temperature and low relative humidity

Iodine-treated filter 1 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.4	34	0	1	1248
40	3.4	32	0	2	1812
60	3.4	32	0	3	3096
80	3.5	32	0	4	5712
100	3.5	33	0	5	28068
120	3.5	32	0	6	58248
Experiment 2.		Room Temperature: 23±2 °C			
20	3.5	36	0	1	888
40	3.6	32	0	2	1860
60	3.5	32	1	3	2724
80	3.5	33	0	4	4920
100	3.5	33	0	5	35616
120	3.5	33	0	6	48552
Experiment 3.		Room Temperature: 23±2 °C			
20	3.4	38	0	1	960
40	3.4	33	1	2	1572
60	3.5	32	1	3	2904
80	3.4	33	0	4	11628
100	3.4	33	0	5	44580
120	3.6	32	0	6	44304
Experiment 4.		Room Temperature: 23±2 °C			
20	3.4	38	1	1	792
40	3.4	33	0	2	936
60	3.5	32	0	3	1884
80	3.4	33	0	4	3024
100	3.4	33	0	5	44580
120	3.6	32	0	6	35616
Experiment 5		Room Temperature: 23±2 °C			
20	3.6	35	0	1	408
40	3.6	34	0	2	936
60	3.6	32	0	3	1428
80	3.7	32	0	4	3228
100	3.7	33	0	5	42960
120	3.6	34	0	6	29520

* CFU is the number of microorganism normalized to 120 minutes.

Iodine-treated filter 2 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	34	0	1	552
40	3.0	33	1	2	612
60	3.0	33	0	3	1212
80	3.0	33	0	4	2388
100	3.0	33	0	5	31344
120	3.2	33	0	6	44016
Experiment 2.		Room Temperature: 23±2 °C			
20	3.0	40	0	1	792
40	3.0	38	0	2	948
60	3.0	34	0	3	1644
80	3.0	34	0	4	2616
100	3.0	33	0	5	33372
120	3.2	33	0	6	25884
Experiment 3.		Room Temperature: 23±2 °C			
20	3.0	38	0	1	360
40	3.1	36	0	2	432
60	3.0	33	0	3	1008
80	3.0	32	1	4	2064
100	3.0	32	0	5	30192
120	3.0	32	0	6	31200
Experiment 4.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	264
40	3.0	38	0	2	564
60	3.0	35	0	3	780
80	3.0	33	0	4	2088
100	3.0	33	0	5	30144
120	3.1	33	0	6	25944
Experiment 5		Room Temperature: 23±2 °C			
20	3.0	39	0	1	516
40	3.0	37	0	2	900
60	3.0	34	0	3	1200
80	3.0	33	0	4	2484
100	3.0	32	0	5	34548
120	3.3	32	0	6	31752

Iodine-treated filter 3 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.3	39	0	1	204
40	3.3	34	0	2	420
60	3.4	33	0	3	600
80	3.4	32	0	4	1380
100	3.4	32	0	5	25536
120	3.4	32	0	6	21192
Experiment 2.		Room Temperature: 23±2 °C			
20	3.4	40	0	1	492
40	3.4	35	0	2	552
60	3.4	35	0	3	1080
80	3.4	34	0	4	2028
100	3.4	33	0	5	27624
120	3.4	32	1	6	26100
Experiment 3.		Room Temperature: 23±2 °C			
20	3.4	40	0	1	168
40	3.4	40	0	2	348
60	3.4	37	0	3	600
80	3.4	36	0	4	1488
100	3.4	35	0	5	25620
120	3.4	32	0	6	22680
Experiment 4.		Room Temperature: 23±2 °C			
20	3.4	39	0	1	564
40	3.4	36	0	2	1104
60	3.4	34	0	3	1800
80	3.4	34	0	4	3408
100	3.4	33	0	5	30468
120	3.4	35	0	6	40368
Experiment 5		Room Temperature: 23±2 °C			
20	3.4	40	0	1	360
40	3.4	40	0	2	660
60	3.4	37	0	3	1116
80	3.4	36	0	4	2400
100	3.4	35	0	5	31860
120	3.4	35	0	6	35436

Untreated filter 1 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	38	0	1	720
40	3.0	36	0	2	828
60	3.0	34	0	3	1260
80	3.0	33	0	4	2472
100	3.0	33	0	5	36264
120	3.0	33	0	6	33324
Experiment 2.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	684
40	3.0	37	0	2	1008
60	3.0	34	0	3	1632
80	3.0	33	0	4	3060
100	3.0	33	0	5	42960
120	3.0	32	0	6	37884
Experiment 3.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	660
40	3.0	34	1	2	912
60	3.0	33	0	3	1380
80	3.0	33	0	4	2700
100	3.0	33	0	5	32292
120	3.2	33	0	6	26484
Experiment 4.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	252
40	3.0	35	0	2	396
60	3.0	35	0	3	492
80	3.0	34	0	4	1560
100	3.0	34	0	5	24672
120	3.2	33	0	6	20604
Experiment 5		Room Temperature: 23±2 °C			
20	3.0	40	0	1	408
40	3.0	38	0	2	936
60	3.0	36	1	3	1428
80	3.0	34	0	4	3228
100	3.0	33	0	5	34092
120	3.0	33	0	6	27060

Untreated filter 2 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	40	0	1	288
40	3.0	40	0	2	552
60	3.0	38	0	3	612
80	3.0	34	0	4	1368
100	3.0	34	0	5	28644
120	3.0	33	0	6	20724
Experiment 2.		Room Temperature: 23±2 °C			
20	3.0	35	0	1	120
40	3.0	35	0	2	252
60	3.1	35	0	3	648
80	3.2	34	0	4	960
100	3.2	33	0	5	19788
120	3.2	33	0	6	20484
Experiment 3.		Room Temperature: 23±2 °C			
20	3.1	38	1	1	480
40	3.0	33	1	2	816
60	3.0	33	0	3	1260
80	3.0	33	0	4	2412
100	3.0	33	0	5	29232
120	3.0	33	0	6	28572
Experiment 4.		Room Temperature: 23±2 °C			
20	3.0	40	0	1	168
40	3.0	40	0	2	672
60	3.1	38	0	3	1044
80	3.0	36	0	4	2088
100	3.0	33	0	5	30600
120	3.2	33	0	6	35472
Experiment 5		Room Temperature: 23±2 °C			
20	3.1	40	0	1	384
40	3.1	36	0	2	768
60	3.1	34	0	3	1116
80	3.2	33	2	4	2148
100	3.1	34	0	5	29472
120	3.1	34	0	6	22608

Untreated filter 3 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	53.0	0	1	300
40	3.1	53	0	2	456
60	3.1	52	0	3	636
80	3.2	49	0	4	1296
100	3.2	48	0	5	27684
120	3.1	46	0	6	36816
Experiment 2.		Room Temperature: 23±2 °C			
20	3.1	40	0	1	144
40	3.1	39	0	2	372
60	3.1	39	0	3	780
80	3.1	39	0	4	1392
100	3.2	38	0	5	28068
120	3.1	38	0	6	29412
Experiment 3.		Room Temperature: 23±2 °C			
20	3.1	40	0	1	276
40	3.1	40	1	2	552
60	3.1	37	0	3	588
80	3.1	36	0	4	2112
100	3.2	35	0	5	25200
120	3.1	36	0	6	30348
Experiment 4.		Room Temperature: 23±2 °C			
20	3.1	37	0	1	276
40	3.1	37	0	2	324
60	3.2	35	0	3	480
80	3.1	35	0	4	1284
100	3.1	36	0	5	24924
120	3.1	36	0	6	21528
Experiment 5		Room Temperature: 23±2 °C			
20	3.1	40	0	1	456
40	3.1	38	1	2	444
60	3.1	36	0	3	744
80	3.2	35	0	4	2136
100	3.2	36	0	5	30924
120	3.2	36	0	6	25944

Iodine-treated filter 1 (Room temperature & High RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	2.6	100	0	1	300
40	2.7	100	0	2	564
60	2.6	100	0	3	1008
80	2.7	100	0	4	1800
100	2.6	100	0	5	32844
120	2.6	100	0	6	44304
Experiment 2.		Room Temperature: 23±2 °C			
20	2.9	100	0	1	456
40	2.5	99	0	2	684
60	2.4	100	0	3	1176
80	2.7	100	0	4	2076
100	2.8	80	0	5	32712
120	2.7	87	0	6	39900
Iodine-treated filter 2 (Room temperature & High RH)					
Experiment 1.		Room Temperature: 23±2 °C			
20	2.7	95	0	1	516
40	2.8	90	0	2	636
60	2.6	91	0	3	1236
80	2.6	90	0	4	2544
100	2.8	92	0	5	33804
120	2.8	93	0	6	34056
Experiment 2.		Room Temperature: 23±2 °C			
20	2.8	92	1	1	480
40	2.6	86	0	2	804
60	2.6	91	0	3	1128
80	2.8	92	0	4	2220
100	3.0	96	0	5	32712
120	2.8	93	0	6	42312

Iodine-treated filter 1 (High temperature & High RH)					
Experiment 1.		High Temperature : 40±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	100	0	1	420
40	3.5	100	0	2	888
60	3.4	100	0	3	1224
80	3.6	100	0	4	2544
100	3.4	100	0	5	36264
120	3.6	100	0	6	45660
Experiment 2.		High Temperature : 40±2 °C			
20	3.8	92	0	1	660
40	3.4	86	1	2	732
60	3.0	91	0	3	1152
80	2.1	92	0	4	2892
100	2.6	96	0	5	39264
120	2.7	93	0	6	44952
Iodine-treated filter 2 (High temperature & High RH)					
Experiment 1.		High Temperature : 40±2 °C			
20	2.8	100	0	1	1080
40	2.7	100	0	2	1464
60	2.7	100	0	3	2052
80	2.5	100	0	4	3804
100	2.6	96	0	5	36996
120	2.8	98	0	6	47652
Experiment 2.		High Temperature : 40±2 °C			
20	2.7	98	0	1	684
40	2.7	100	0	2	1188
60	3.0	100	0	3	1728
80	2.7	100	0	4	3708
100	2.8	96	0	5	37140
120	2.8	100	0	6	47652

APPENDIX B
PROCEDURES FOR PREPARING PLAQUE ASSAY MEDIA

- MS2 Media

With gentle mixing, 1.0 g tryptone, 0.1 g yeast extract, 0.1 g D-glucose, 0.8 g NaCl, and 0.022 g $CaCl_2$ wre added to a total volume of 100 mL of distilled water in a 250-mL flask. The mixed medium was autoclaved at 121 ^{o}C for 30 mins.

- MS2 Agar Media

With gentle mixing, 3.0 g tryptone, 0.3 g yeast extract, 0.3 g D-glucose, 2.4 g NaCl, 0.066 g $CaCl_2$, and 0.3 g of Bacto-agar were added to a total volume of 300 mL of distilled water in a 500-mL flask. The mixed agar was autoclaved at 121 ^{o}C for 30 mins.

- 1XPBS dilution tube

1.8 g KH_2PO_4, 15.2 g K_2HPO_4, and 85 g NaCl were added to 1L of distilled water to make 10XPBS. 1XPBS was prepared by diluting 10XPBS in distilled water. 9-mL aliquots of 1XPBS were dispensed into 16×150 mm test tubes and autoclaved at 121 ^{o}C for 30 min.

APPENDIX C
RAW DATA OF VIRUS EXPERIMENT

Table C-1. All glass impinger results at various environmental conditions

Iodine-treated filter 1 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	38	10	3350	99.70
60	0.3	39	30	1590	98.11
90	0.3	39	80	18650	99.57
120	0.2	38	120	19500	99.49
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	0	4450	100.00
60	0.3	38	0	10550	100.00
90	0.2	38	300	40000	99.25
120	0.2	37	100	48500	99.80
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	100	16500	99.39
60	0.2	39	120	15000	99.20
90	0.2	39	110	32000	99.66
120	0.2	38	150	34500	99.57
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	100	19000	99.47
60	0.2	38	250	32000	99.22
90	0.2	39	220	31000	99.29
120	0.3	37	150	15600	99.04
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	110	67000	99.84
60	0.2	38	300	61000	99.51
90	0.2	38	170	12600	98.65
120	0.2	37	260	25000	98.96

Iodine-treated filter 2 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	33	14450	99.78
60	0.2	39	8	18350	99.96
90	0.2	39	44	22250	99.80
120	0.2	39	23	24400	99.91
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	15	59500	99.97
60	0.2	39	0	66000	100.00
90	0.2	39	0	36000	100.00
120	0.2	40	100	49500	99.80
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	100	17000	99.41
60	0.2	38	0	6500	100.00
90	0.3	38	150	26000	99.42
120	0.3	37	200	29500	99.32
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	120	56700	99.79
60	0.2	39	400	70000	99.43
90	0.2	38	120	27000	99.56
120	0.2	38	300	20500	98.54
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	90	29500	99.69
60	0.2	39	130	19000	99.32
90	0.2	40	90	32000	99.72
120	0.2	38	110	60000	99.82

Untreated filter 1 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	2320	21700	89.31
60	0.2	40	2115	25600	91.74
90	0.2	39	2405	21200	88.66
120	0.2	38	2370	28350	91.64
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	345	7400	95.34
60	0.2	39	550	11650	95.28
90	0.2	38	570	7800	92.69
120	0.2	38	350	7300	95.21
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	3480	47850	92.73
60	0.2	39	2820	29950	90.58
90	0.2	38	1850	26200	92.94
120	0.2	38	2310	28900	92.01
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	540	6000	91.00
60	0.2	38	270	3550	92.39
90	0.2	38	185	3050	93.93
120	0.2	38	315	4950	93.64
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	680	8350	91.86
60	0.2	39	580	7600	92.37
90	0.2	39	455	6200	92.66
120	0.2	38	700	9000	92.22

Untreated filter 2 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	430	4000	89.25
60	0.2	39	280	4250	93.41
90	0.2	39	445	4400	89.89
120	0.2	38	275	3200	91.41
Experiment 2.		Temperature: 23±2 °C			
30	0.2	39	1660	10650	84.41
60	0.2	39	515	9400	94.52
90	0.2	38	1110	10200	89.12
120	0.2	38	490	5350	90.84
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	915	8450	89.17
60	0.2	39	595	5600	89.38
90	0.2	38	1365	18500	92.62
120	0.2	38	730	9400	92.23
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	1705	17050	90.00
60	0.2	39	805	8350	90.36
90	0.2	39	865	7800	88.91
120	0.2	38	710	9150	92.24
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	1480	22300	93.36
60	0.2	39	1220	15050	91.89
90	0.2	40	535	5250	89.81
120	0.2	38	415	4800	91.35

Iodine-treated filter 1 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.5	56	90	17700	99.49
60	0.3	51	40	10700	99.63
90	0.3	50	20	16900	99.88
120	0.3	53	0	8800	100.00
Experiment 2.		Temperature: 23±2 °C			
30	0.3	60	1	5150	99.98
60	0.2	53	0	5100	100.00
90	0.3	51	3	2650	99.89
120	0.3	56	3	4750	99.94
Experiment 3.		Temperature: 23±2 °C			
30	0.3	57	0	2500	100.00
60	0.2	51	65	6950	99.06
90	0.2	51	55	9350	99.41
120	0.2	51	10	7050	99.86
Experiment 4.		Temperature: 23±2 °C			
30	0.3	49	10	2150	99.53
60	0.3	52	20	2300	99.13
90	0.3	60	20	4200	99.52
120	0.3	57	0	4000	100.00
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	0	3450	100.00
60	0.2	38	0	1100	100.00
90	0.2	38	0	4150	100.00
120	0.2	37	0	2350	100.00

Iodine-treated filter 2 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	58	0	3000	100.00
60	0.3	54	0	6000	100.00
90	0.2	59	0	1850	100.00
120	0.2	56	0	2000	100.00
Experiment 2.		Temperature: 23±2 °C			
30	0.3	56	0	950	100.00
60	0.2	54	0	3650	100.00
90	0.3	57	10	700	98.57
120	0.3	56	10	300	96.67
Experiment 3.		Temperature: 23±2 °C			
30	0.2	57	0	3100	100.00
60	0.3	59	1	2150	99.95
90	0.3	57	0	1400	100.00
120	0.3	59	0	1450	100.00
Experiment 4.		Temperature: 23±2 °C			
30	0.2	55	0	550	100.00
60	0.2	57	0	700	100.00
90	0.2	56	0	1700	100.00
120	0.2	57	0	2050	100.00
Experiment 5.		Temperature: 23±2 °C			
30	0.3	69	0	1750	100.00
60	0.3	59	0	1750	100.00
90	0.3	60	0	2300	100.00
120	0.3	58	0	600	100.00

Untreated filter 1 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	47	1100	43000	97.44
60	0.3	46	900	20000	95.50
90	0.3	45	1000	19000	94.74
120	0.3	45	1300	21000	93.81
Experiment 2.		Temperature: 23±2 °C			
30	0.3	43	6350	121000	94.75
60	0.2	47	10100	178000	94.32
90	0.3	46	9050	101500	91.08
120	0.3	45	9950	245000	95.94
Experiment 3.		Temperature: 23±2 °C			
30	0.3	46	4150	41000	89.88
60	0.3	45	4300	44000	90.23
90	0.3	46	4150	55000	92.46
120	0.3	46	3150	72000	95.63
Experiment 4.		Temperature: 23±2 °C			
30	0.3	51	1000	14050	92.88
60	0.3	49	685	12400	94.48
90	0.3	47	925	14550	93.64
120	0.3	48	1000	17500	94.29
Experiment 5.		Temperature: 23±2 °C			
30	0.3	50	3050	33500	90.90
60	0.3	46	1800	22500	92.00
90	0.3	45	1600	26500	93.96
120	0.3	44	2900	30000	90.33

Untreated filter 2 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	48	3150	33000	90.46
60	0.2	47	1850	21150	91.25
90	0.2	47	1650	16200	89.82
120	0.2	47	750	16700	95.51
Experiment 2.		Temperature: 23±2 °C			
30	0.2	47	1000	14800	93.24
60	0.2	48	1650	15200	89.15
90	0.2	47	1900	19000	90.00
120	0.2	45	1100	19000	94.21
Experiment 3.		Temperature: 23±2 °C			
30	0.2	49	2650	28500	90.70
60	0.2	47	3600	39000	90.77
90	0.2	47	2650	35500	92.54
120	0.2	45	3350	31500	89.37
Experiment 4.		Temperature: 23±2 °C			
30	0.2	47	1300	16350	92.05
60	0.2	49	1350	16250	91.69
90	0.2	45	1900	13500	85.93
120	0.2	45	1700	24200	92.98
Experiment 5.		Temperature: 23±2 °C			
30	0.2	47	3250	39500	91.77
60	0.2	47	4050	44000	90.80
90	0.2	44	2100	25500	91.77
120	0.2	45	3700	41500	91.08

Iodine-treated filter 1 (High temperature & Low RH)					
Experiment 1.		Temperature: 40±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	39	0	800	100.00
60	0.3	38	0	445	100.00
90	0.3	38	0	26500	100.00
120	0.3	40	0	38000	100.00
Experiment 2.		Temperature: 40±2 °C			
30	0.3	40	0	47500	100.00
60	0.3	39	0	43500	100.00
90	0.3	39	0	40500	100.00
120	0.3	38	0	41000	100.00
Experiment 3.		Temperature: 40±2 °C			
30	0.3	39	0	53500	100.00
60	0.3	38	0	50000	100.00
90	0.3	38	0	57500	100.00
120	0.3	38	0	58500	100.00
Experiment 4.		Temperature: 40±2 °C			
30	0.3	39	0	54500	100.00
60	0.3	39	0	62000	100.00
90	0.3	40	0	28500	100.00
120	0.3	38	0	40500	100.00
Experiment 5.		Temperature: 40±2 °C			
30	0.3	39	0	16000	100.00
60	0.3	40	0	19500	100.00
90	0.3	39	0	18500	100.00
120	0.3	39	0	17500	100.00

Iodine-treated filter 2 (High temperature & Low RH)					
Experiment 1.		Temperature: 30±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	39	1	8300	99.99
60	0.3	38	0	6150	100.00
90	0.3	39	1	9200	99.99
120	0.3	34	0	11300	100.00
Experiment 2.		Temperature: 30±2 °C			
30	0.3	42	1	5150	99.98
60	0.2	41	0	5100	100.00
90	0.3	37	1	2650	99.96
120	0.3	32	3	4750	99.94
Experiment 3.		Temperature: 30±2 °C			
30	0.2	40	0	20000	100.00
60	0.2	32	1	17000	99.99
90	0.2	36	1	14000	99.99
120	0.2	38	1	21500	99.99
Experiment 4.		Temperature: 30±2 °C			
30	0.3	34	0	2800	100.00
60	0.3	35	0	1550	100.00
90	0.3	35	0	5950	100.00
120	0.3	35	0	4000	100.00
Experiment 5.		Temperature: 30±2 °C			
30	0.3	34	4	3500	99.89
60	0.3	36	1	500	99.80
90	0.3	46	0	2500	100.00
120	0.3	35	1	4450	99.98

Untreated filter 1 (High temperature & Low RH)					
Experiment 1.		Temperature: 30±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	33	3550	101000	96.49
60	0.3	35	6900	74500	90.74
90	0.3	40	3950	102500	96.15
120	0.3	39	4850	119000	95.92
Experiment 2.		Temperature: 30±2 °C			
30	0.3	36	2850	87500	96.74
60	0.2	35	3100	137500	97.75
90	0.3	33	6950	50000	86.10
120	0.3	35	8650	90000	90.39
Experiment 3.		Temperature: 30±2 °C			
30	0.3	40	580	14000	95.86
60	0.3	39	1470	18000	91.83
90	0.3	43	1135	14000	91.89
120	0.3	35	825	14500	94.31
Experiment 4.		Temperature: 30±2 °C			
30	0.4	27	2850	155000	98.16
60	0.2	33	5450	90000	93.94
90	0.3	34	4200	134500	96.88
120	0.3	30	5550	35000	84.14
Experiment 5.		Temperature: 30±2 °C			
30	0.2	41	3400	99500	96.58
60	0.2	40	3400	80500	95.78
90	0.2	33	3000	112000	97.32
120	0.2	37	6000	97000	93.80

Untreated filter 2 (High temperature & Low RH)					
Experiment 1.		Temperature: 30±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	38	2335	16500	85.85
60	0.3	36	360	27000	98.67
90	0.3	38	1705	16000	89.34
120	0.3	37	2430	35000	93.06
Experiment 2.		Temperature: 30±2 °C			
30	0.3	42	1785	29500	93.95
60	0.3	42	895	33500	97.33
90	0.3	43	1065	31500	96.62
120	0.3	38	2210	19000	88.37
Experiment 3.		Temperature: 30±2 °C			
30	0.3	38	2050	34500	94.06
60	0.2	32	1750	29000	93.97
90	0.3	35	2150	32500	93.38
120	0.3	42	2400	35000	93.14
Experiment 4.		Temperature: 30±2 °C			
30	0.3	34	1200	14000	91.43
60	0.3	38	950	8500	88.82
90	0.3	40	850	12500	93.20
120	0.3	39	950	15000	93.67
Experiment 5.		Temperature: 30±2 °C			
30	0.3	25	3050	36500	91.64
60	0.3	29	2900	28500	89.82
90	0.3	39	3000	15000	80.00
120	0.3	40	2100	11500	81.74

APPENDIX D
PARTICLE SIZE DISTRIBUTION OF NUMBER-BASED, MASS-BASED, AND INFECTIOUS VIRUSES

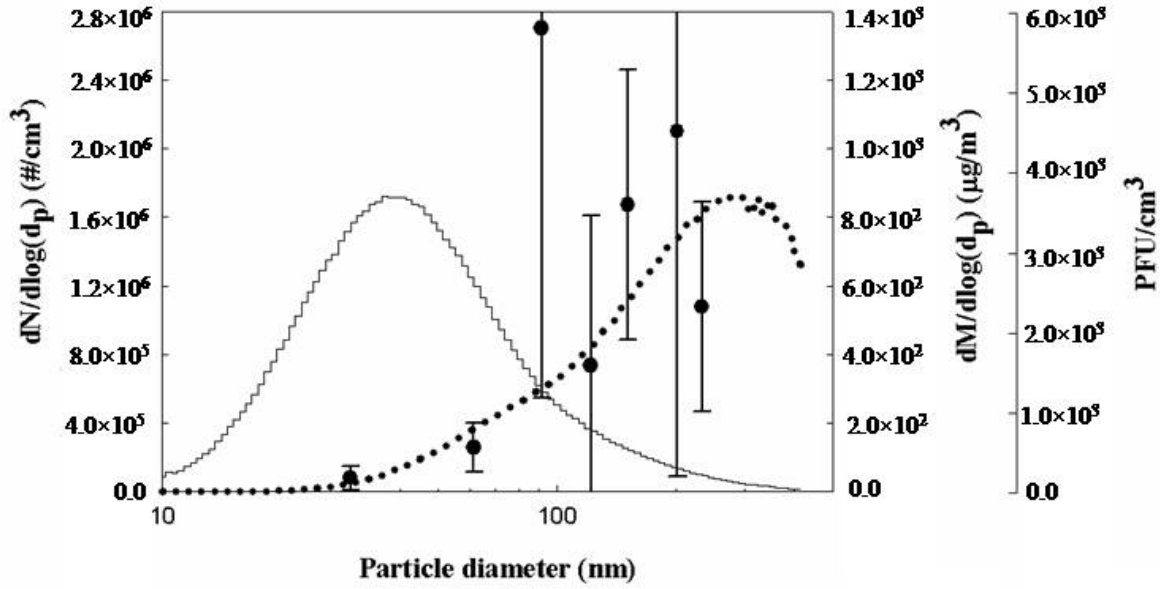


Figure D-1. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from sterile DI water at medium relative humidity.

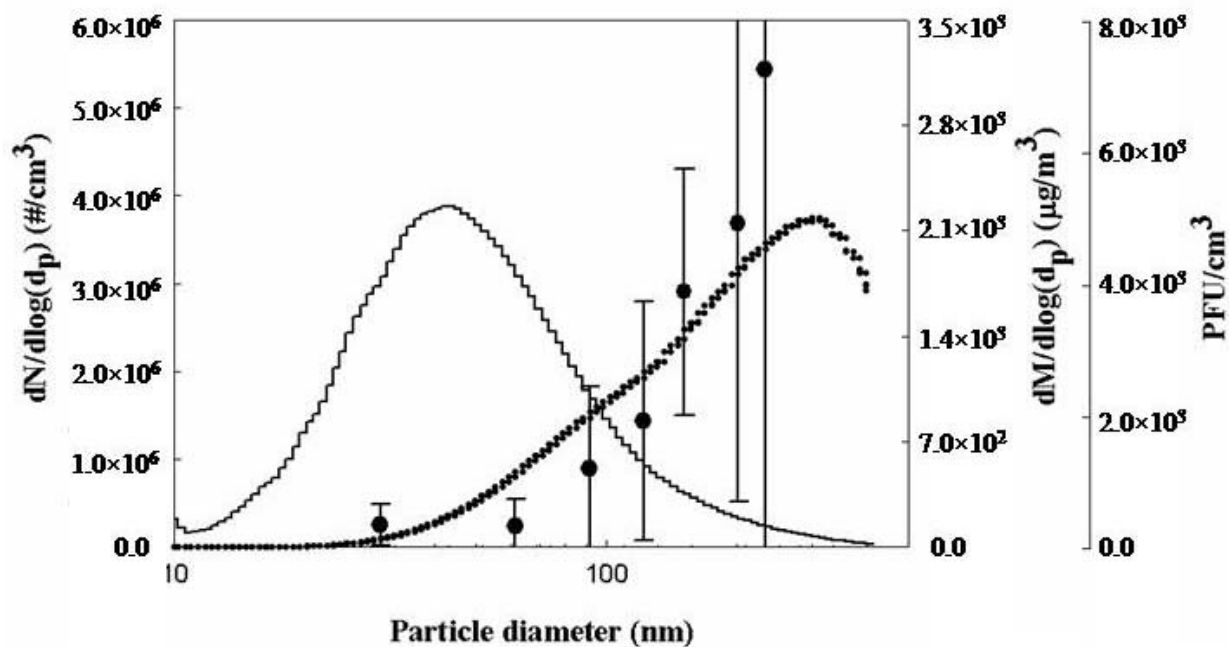


Figure D-2. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from sterile DI water at high relative humidity.

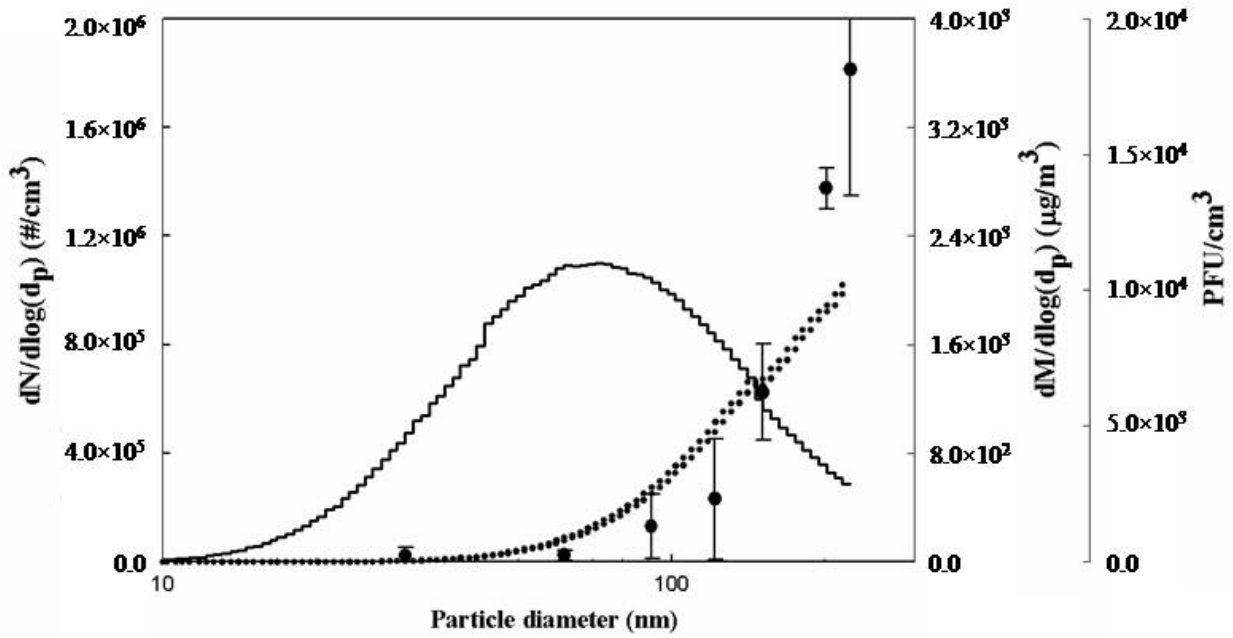


Figure D-3. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from tryptone solution at medium relative humidity.

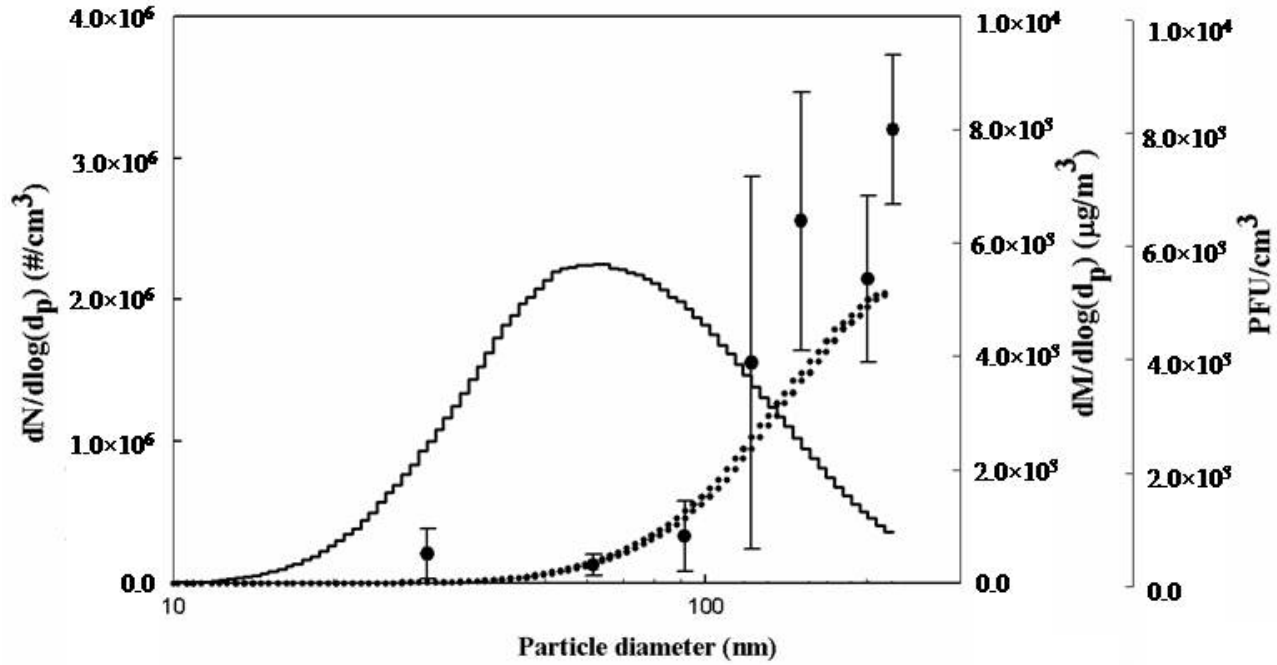


Figure D-4. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from tryptone solution at high relative humidity.

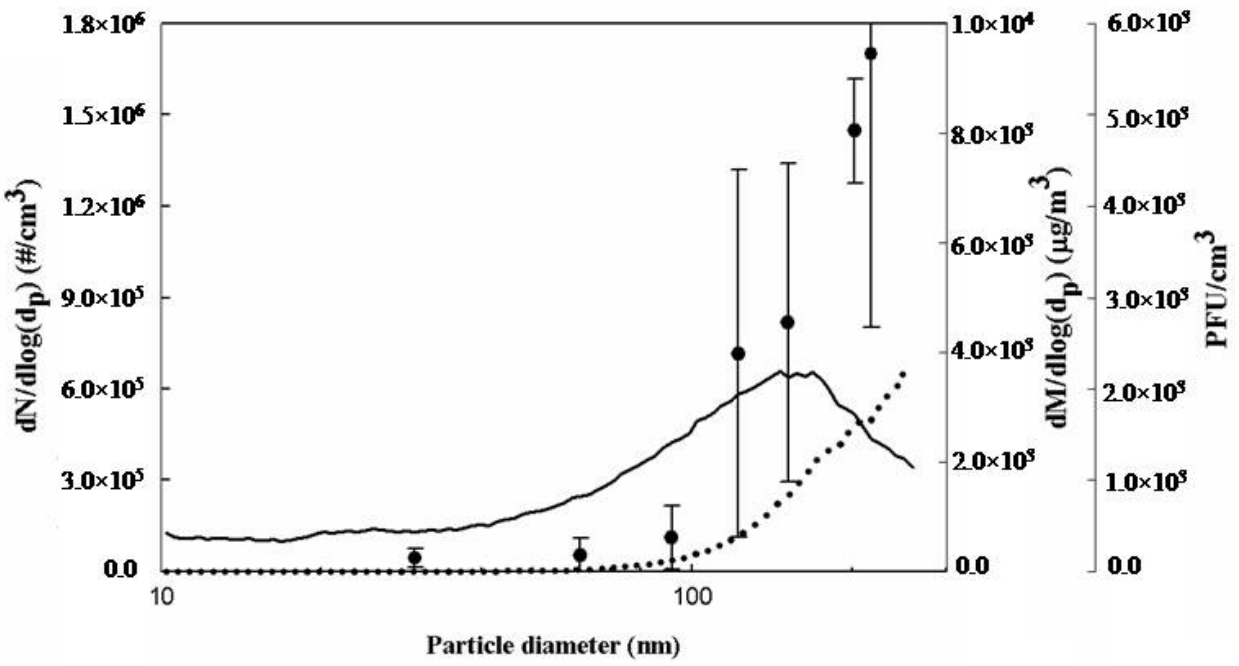


Figure D-5. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from artificial saliva at medium relative humidity.

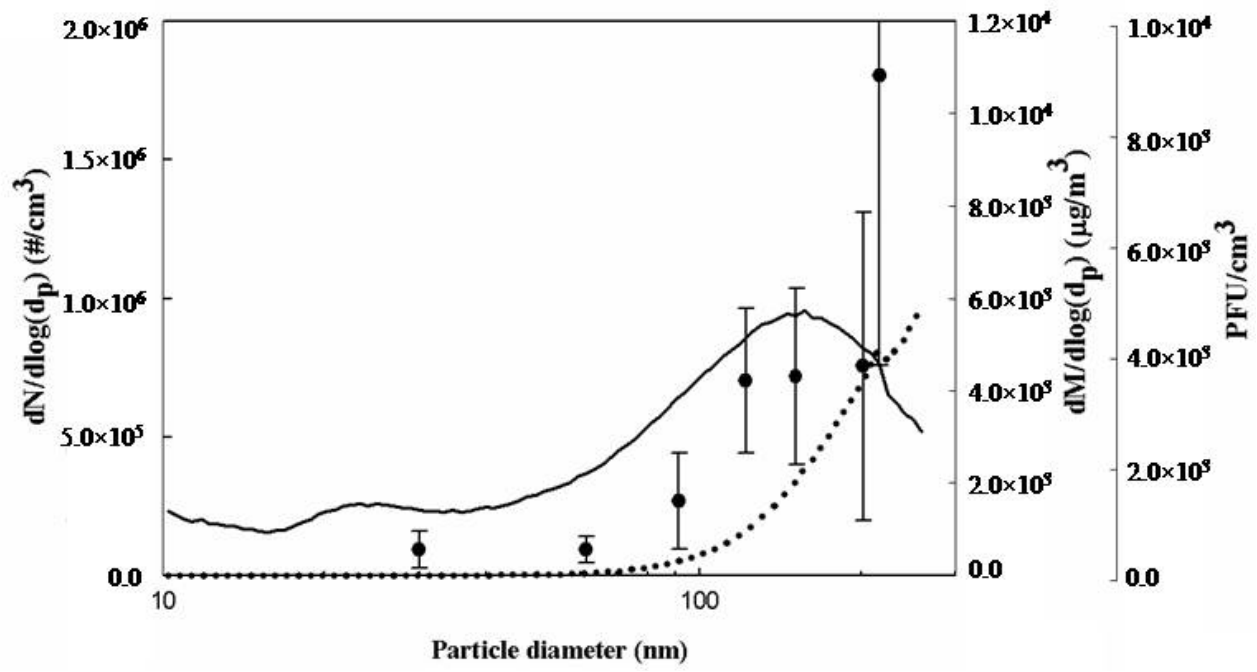


Figure D-6. Particle size distribution by number (solid line), mass (dotted line), and infectious count (mean with error bars) for MS2 aerosols generated from artificial saliva at high relative humidity.

APPENDIX E
RAW DATA OF CHARACTERIZATION EXPERIMENT

Table E-1. Bioassay results (plaque-forming units)

MS2 aerosols generated from sterile DI water			
Low RH (25±5%)			
Particle diameter (nm)	Set 1	Set 2	Set 3
30	2963	1200	4500
60	1028	285	510
90	1238	2700	2980
120	1043	3375	3413
150	2483	4222	4118
200	2558	7050	9375
230	14850	15675	10800
Medium RH (45±5%)			
30	8	353	53
60	98	143	30
90	–	645	315
120	45	630	240
150	330	135	315
200	585	75	–
230	135	540	420
High RH (85±5%)			
30	–	255	53
60	23	540	38
90	45	555	–
120	180	540	45
150	585	810	270
200	570	1605	225
230	315	2625	180
MS2 aerosols generated from tryptone solution			
Low RH (25±5%)			
Particle diameter (nm)	Set 1	Set 2	Set 3
30	120	225	45

60	75	435	75
90	120	990	105
120	300	675	120
150	435	735	60
200	1320	1170	870
230	2520	2355	2220
Medium RH (45±5%)			
30	15	255	45
60	60	300	120
90	120	570	120
120	105	675	690
150	855	1200	1800
200	1215	2400	2220
230	1905	2985	3195
High RH (85±5%)			
30	45	450	210
60	270	300	270
90	330	300	330
120	1005	525	1005
150	1320	750	1320
200	1050	1035	1050
230	1035	1410	1035

MS2 aerosols generated from artificial saliva		
Low RH (25±5%)		
Particle diameter (nm)	Set 1	Set 2
30	45	105
60	75	120
90	30	150
120	90	225
150	735	150
200	615	240
230	1125	1485

Medium RH (45±5%)		
30	225	135
60	525	150
90	990	330
120	1215	675
150	1365	660
200	1485	1755
230	2505	1815
High RH (85±5%)		
30	675	495
60	765	885
90	990	975
120	1185	1185
150	1335	1305
200	1815	1800
230	3450	3705

Table E-2. Polymerase chain reaction results (*ng*)

MS2 aerosols generated from sterile DI water						
Particle diameter (nm)	Low RH		Medium RH		High RH	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
30	0.60	1.27	0.40	1.22	–	–
90	–	–	–	–	0.29	0.42
120	0.50	1.87	0.60	0.18	0.81	0.40
200	0.63	1.91	0.52	1.72	1.05	1.73
MS2 aerosols generated from tryptone solution						
30	0.17	1.18	0.33	2.70	–	7.39
90	–	–	–	–	0.67	2.68
120	0.70	2.71	1.19	1.70	0.57	3.28
200	0.65	7.01	0.72	4.28	0.79	4.50
MS2 aerosols generated from artificial saliva						
30	0.29	0.25	0.69	0.62	0.63	0.94
120	1.26	1.31	1.40	1.69	1.69	2.27
200	2.38	2.94	2.52	6.20	7.25	8.66

LIST OF REFERENCES

ACGIH (2001) Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adams, M. H. (1948) Surface inactivation of bacterial viruses and of proteins. *J Gen Physiol* **31,** 417-431.

Alvarez, A. J., Buttner, M. P. and Stetzenbach, L. D. (1995) PCR for bioaerosol monitoring: sensitivity and environmental interference. *Appl Environ Microbiol* **61** (10)**,** 3639-3644.

Alvarez, M. E., M. Aguilar, A. Fountain, N. Gonzalez, O. Rascon and Saenz., D. (2000) Inactivation of MS-2 phage and poliovirus in groundwater. *Can J Microbiol* **46,** 159-165.

APHA (1995) Standard methods for the examination of water and wastewater, Method 4500 CI G. Washington, DC: American Public Health Association.

Aps, J. K. M. and Martens, L. C. (2005) Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* **150,** 119-131.

Aranha-Creado, H. and Brandwein, H. (1999) Application of bacteriophages as surrogates for mammalian viruses: A case for use in filter validation based on precedents and current practices in medical and environmental virology. *PDA J Pharm Sci Technol* **53** (2)**,** 75-82.

ATCC (1998) Methods for enhancing *Bacillus* sp. spore production. Manassas, VA: American Type Culture Collection.

ATSDR (2004) Toxicological profile for iodine. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Balazy, A., Toivola, M., Adhikari, A., Sivasubramani, S. K., Reponen, T. and Grinshpun, S. A. (2006) Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks? *Am J Infect Control* **34** (2)**,** 51-57.

Barlow, D. F. and Donaldson, A. I. (1973) Comparison of the aerosol stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. *J Gen Virol* **20,** 311-318.

Baron, P. A. and Willeke, K. (2001) Aerosol measurement: principles, techniques, and applications, New York, NY: John Wiley & Sons, Inc.

Barrett, L. W. and Rousseau, A. D. (1998) Aerosol loading performance of electret filter media. *Am Ind Hyg Assoc J* **59,** 532–539.

Beggs, C. B. (2003) The airborne transmission of infection in hospital buildings: fact or fiction? *Indoor and Built Environ* **12** (1-2)**,** 9-18.

Belgrader, P., Benett, W., Hadley, D., Long, G., Mariella, J., R., Milanovich, F., Nasarabadi, S., Nelson, W., Richards, J. and Strattion, P. (1998) Rapid pathogen detection using a microchip PCR array instrument. *Clin Chem* **44,** 2191-2194.

Benbough, J. E. (1969) The effect of relative humidity on the survival of airborne Semliki Forest virus. *J Gen Virol* **4,** 473-477.

Benbough, J. E. (1971) Some factors affecting the survival of airborne viruses. *J Gen Virol* **10,** 209-220.

Berg, G., Chang, S. L. and Harris, E. K. (1964) Devitalization of microorganisms by iodine. *Virol* **22,** 469-481.

Bergey, E. J., Cho, M. I., Blumberg, B. M., Hammarskjold, M. L., Rekosh, D., Epstein, L. G. and Levine, M. J. (1994) Inhibition of HIV-1 with human salivary mucins. *J Acquir Immune Defic Syndr* **7,** 995-1002.

Bergey, E. J., Gu, M., Collins, A. R., Bradway, S. D. and Levine, M. J. (1993) Modulation of herpes-simplex virus type-1 replication by human salivary secretions. *Oral Microbiol Immun* **8** (2)**,** 89-93.

Biswas, P. and Wu, C. Y. (2005) Critical Review: Nanoparticles and the environment. *J Air Waste Manag Assoc* **55** (6)**,** 708-746.

Brion, G. M. and Silverstein, J. (1999) Iodine disinfection of a model bacteriophage, MS2, demonstrating apparent rebound. *Water Res* **33** (1)**,** 169-179.

Brosseau L. M., McCullough N.V. and Vesley, D. (1997) Bacterial survival on respirator filters and surgical masks. *J Am Biol Saf Assoc* **2,** 32-43.

Bruchertseifer, H., Cripps, R., Guentay, S. and Jaeckel, B. (2003) Analysis of iodine species in aqueous solutions. *Anal Bioanal Chem* (375)**,** 1107-1110.

Cameron, M. (2002) Iodine: Inhalation hazards, detection and protection. Sacramento, CA: California Criminalistics Institute.

Carroll, B. (1955) The relative germicidal activity of triiodide and diatomic iodine. *J Bacteriol* **69,** 413-417.

CDC (2004) Molecular subtyping of *bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, united states. Atlanta, GA: Center for Disease Control and Prevention. Available at: http://www.cdc.gov/ncidod/eid/vol8no10/02-0394.htm.

CDC (2005) Fact sheet: Basic Information about SARS. Atlanta, GA: Center for Disease Control and Prevention.

CDC (2009) H1N1 Flu (Swine Flu). Atlanta, GA: Center for Disease Control and Prevention. Available at: http://www.cdc.gov/h1n1flu/.

Cecchini, C., Verdenelli, M. C., Orpianesi, C., Dadea, G. M. and Cresci, A. (2004) Effects of antimicrobial treatment on fiberglass-acrylic filters. *J Appl Microbiol* **97** (2)**,** 371-377.

CFR (2002) Respiratory Protective Devices, 42 CFR Part 84. Washington DC: U.S. Government Printing Office.

Chang, J. C. S., Foarde, K. K. and Vanosdell, D. W. (1995) Growth evaluation of fungi (*Penicillium* and *Aspergillus* Spp) on ceiling tiles. *Atmos Environ* **29** (17)**,** 2331-2337.

Chang, S. L. (1958) The use of active iodine as a water disinfectant. *J Am Pharm Assoc* **47,** 417-423.

Cox, C. S. (1989) Airborne bacteria and viruses. *Sci Prog Oxf* **73,** 469-500.

Cox, C. S. (1995) Stability of airborne microbes and allergens. In *Bioaerosols Handbook*. Cox, C. S. and Wathes, C. M. eds., Boca Raton, FL: CRC Press LLC.

Di Ionno, L. and Messier, P. J. (2004) Iodinated thermal-fused polymer as a biocidal additive to commercial and chemical-resistant coatings. *Surf Coatings Int Part B: Coatings Transactions* **87,** 235-308.

Dubovi, E. J. (1971) Biological activity of the nucleic acids extracted from two aerosolized bacterial viruses. *Appl Microbiol* **21,** 761-762.

Dubovi, E. J. and Akers, T. G. (1970) Airborne stability of tailless bacterial viruses S-13 and MS-2. *Appl Microbiol* **19,** 624-628.

Ehrlich, R., Miller, S. and Idoine, L. S. (1964) Effects of environmental factors on survival of airborne T-3 coliphage. *Appl Microbiol* **12** (6)**,** 479-482.

Eninger, R. M., Adhikari, A., Reponen, T. and Grinshpun, S. A. (2008a) Differentiating between physical and viable penetrations when challenging respirator filters with bioaerosols. *Clean* **36** (7)**,** 615–621.

Eninger, R. M., Honda, T., Adhikari, A., Heinonen-Tanski, H., Reponen, T. and Grinshpun, S. A. (2008b) Filter performance of N99 and N95 facepiece respirators against viruses and ultrafine particles. *Ann Occup Hyg* **52** (5)**,** 385-396.

Ezeonu, I. M., Noble, J. A., Simmons, R. B., Price, D. L., Crow, S. A. and Ahearn, D. G. (1994) Effect of relative-humidity on fungal colonization of fiberglass insulation. *Appl Environ Microbiol* **60** (6)**,** 2149-2151.

Fiegel, J., Clarke, R. and Edwards, D. A. (2006) Airborne infectious disease and the suppression of pulmonary bioaerosols. *Drug Discov Today* **11** (1-2)**,** 51-57.

Fiers, W., Contreras, R., Duerinck, F., Haegeman, G., Iserentant, D., Merregaert, J., Minjou, W., Molemans, F., Raeymaekers, A., Vandenberghe, A., Volckaert, G. and Ysebaert, M. (1976) Complete nucleotide-sequence of bacteriophage MS2 RNA - primary and secondary structure of replicase gene. *Nature* **260** (5551)**,** 500-507.

Fina, L. R., Hassouna, N., Horacek, G. L., Lambert, J. P. and Lambert, J. L. (1982) Viricidal capability of resin-triiodide demand-type disinfectant. *Appl Environ Microbiol* **44** (6)**,** 1370-1373.

Foarde, K. K., Hanley, J. T. and Veeck, A. C. (2000) Efficacy of antimicrobial filter treatments. *ASHRAE J* **42** (12)**,** 52-58.

Foarde, K. K., VanOsdell, D. W. and Chang, J. C. S. (1996) Evaluation of fungal growth on fiberglass duct materials for various moisture, soil, use, and temperature conditions. *Indoor Air-Int J Indoor Air Qual Climate* **6** (2)**,** 83-92.

Fox, P. C., Wolff, A., Yeh, C. K., Atkinson, J. C. and Baum, B. J. (1988) Saliva inhibits HIV-1 Infectivity. *J Am Dent Assoc* **116** (6)**,** 635-637.

Galasso, G. J. and Sharp, D. G. (1965) Effect of particle aggregation on survival of irradiated vaccinia virus. *J Bacteriol* **90** (4)**,** 1138-1142.

Gilmour, M. N. and Wicksell, E. K. (1972) Bactericidal and sporicidal activity of a quaternary ammonium resin-triiodide complex. *Antimicrob Agents Ch* **22** (5)**,** 417-418.

Gottardi, W. (2001) Iodine and Iodine Compounds. In *Disinfection, Sterilization, and Preservation*. Block, S. S. eds., New York, NY: Lippincott Williams & Wilkins.

Greenwood, F. C., Hunter, W. M. and Glover, J. S. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem J* **89,** 114–123.

Habte, H. H., Mall, A. S., de Beer, C., Lotz, Z. E. and Kahn, D. (2006) The role of crude human saliva and purified salivary MUC5B and MUC7 mucins in the inhibition of Human Immunodeficiency Virus type 1 in an inhibition assay. *Virol J* **3,** doi: 10.1186/1743-422X-3-99.

Hach (2003) Iodine Method 8031. Loveland, CO: Hach Company.

Harper, G. J. (1961) Airborne microorganisms: survival tests with four viruses. *J Hyg (London)* **59** (4)**,** 479-486.

Hartshorn, K. L., Ligtenberg, A., White, M. R., van Eijk, M., Hartshorn, M., Pemberton, L., Holmskov, U. and Crouch, E. (2006) Salivary agglutinin and lung scavenger receptor

cysteine-rich glycoprotein 340 have broad anti-influenza activities and interactions with surfactant protein D that vary according to donor source and sialylation. *Biochem J* **393,** 545-553.

Hatch, G. L., Lambert, J. L. and Fina, L. R. (1980) Some properties of the quaternary ammonium anion-exchange resin-triiodide disinfectant for water. *Ind Eng Chem Prod RD* **19** (2)**,** 259-263.

Heimbuch, B. K., Hodge, J. E. and Wander, J. D. (2007) Viral penetration of high efficiency particulate air (HEPA) filters. Tyndall AFB, FL: Air Force Research Laboratory.

Heimbuch, B. K. and Wander, J. D. (2006) Bioaerosol challenges to antimicrobial surface treatments: enhanced efficacy against MS2 coli phage of air filter media coated with polystyrene-4-methyltrimethylammonium triiodide. Tyndall AFB, FL: Air Force Research Laboratory.

Heldal, K., Skogstad, A. and Eduard, W. (1996) Improvements in the quantification of airborne microorganisms in the farm environment by epifluorescence microscopy. *Ann Occup Hyg* **40** (4)**,** 437-447.

Henderson, D. A. (1999) The looming threat of bioterrorism. *Science* **283** (5406)**,** 1279-1282.

Hermann, J., Hoff, S., Munoz-Zanzi, C., Yoon, K. J., Roof, M., Burkhardt, A. and Zimmerman, J. (2007) Effect of temperature and relative humidity on the stability of infectious porcine reproductive and respiratory syndrome virus in aerosols. *Vet Res* **38** (1)**,** 81-93.

Hinds, W. C. (1999a) Bioaerosols, In *Aerosol Technology*. New York, NY: John Wiley & Sons, Inc.

Hinds, W. C. (1999b) Filtration, In *Aerosol Technology*. New York, NY: John Wiley & Sons, Inc.

Hinds, W. C. (1999c) Production of Test Aerosols, In *Aerosol Technology*. New York, NY: John Wiley & Sons, Inc.

Hinds, W. C. (1999d) Uniform Particle Motion, In *Aerosol Technology*. New York, NY: John Wiley & Sons, Inc.

Hogan, C. J., Kettleson, E. M., Lee, M. H., Ramaswami, B., Angenent, L. T. and Biswas, P. (2005) Sampling methodologies and dosage assessment techniques for submicrometre and ultrafine virus aerosol particles. *J Appl Microbiol* **99** (6)**,** 1422-1434.

Hogan, C. J., Lee, M. H. and Biswas, P. (2004) Capture of viral particles in soft X-ray-enhanced corona systems: Charge distribution and transport characteristics. *Aerosol Sci Technol* **38** (5)**,** 475-486.

Hsu, Y. C. (1964) Resistance of infectious RNA + transforming DNA to iodine which inactivates F2 phage + cells. *Nature* **203** (494)**,** 152-153.

Hsu, Y. C., Nomura, S. and Kruse, C. W. (1965) Some bactericidal and virucidal properties of iodine not affecting infectious RNA and DNA. *Am J Epidemiol* **82** (3)**,** 317-328.

Hugenholtz, P. and Fuerst, J. A. (1992) Heterotrophic bacteria in an air-handling system. *Appl Environ Microbiol* **58** (12)**,** 3914-3920.

Hughes, W. L. J. and Straessle, R. (1950) Preparation and properties of serum and plasma proteins. XXIV. Iodination of human serum albumin. *J Am Chem Soc* **72,** 452–457.

Ijaz, M. K., Sattar, S. A., Johnsonlussenburg, C. M., Springthorpe, V. S. and Nair, R. C. (1985) Effect of relative humidity, atmospheric temperature, and suspending medium on the airborne survival of human rotavirus. *Can J Microbiol* **31** (8)**,** 681-685.

Jacoby, W. A., Maness, P. C., Wolfrum, E. J., Blake, D. M. and Fennel, J. A. (1998) Mineralization of bacteria cell mass on a photocatalytic surface in air. *Environ Sci Technol* **32** (17)**,** 2650-2653.

Jankowska, E., Reponen, T., Willeke, K., Grinshpun, S. A. and Choi, K. J. (2000) Collection of fungal spores on air filters and spore reentrainment from filters into air. *J Aerosol Sci* **31** (8)**,** 969-978.

Kemp, S. J., Kuehn, T. H., Pui, D. Y. H. and Vesley, D. (1995a) Growth of microorganisms on HVAC filters under controlled temperature and humidity conditions. *ASHRAE Trans* **101,** 305-316.

Kemp, S. J., Kuehn, T. H., Pui, D. Y. H., Vesley, D. and Streifel, A. J. (1995b) Filter collection efficiency and growth of microorganisms on filters loaded with outdoor air. *ASHRAE Trans* 228-238.

Kowalski, W. J. and Bahnfleth, W. (1998) Airborne respiratory diseases and mechanical systems for control of microbes. *HPAC Engineering*.

Kuzmanovic, D. A., Elashvili, L., Wick, C., O'Connell, C. and Krueger, S. (2003) Bacteriophage MS2: Molecular weight and spatial distribution of the protein and RNA components by small-angle neutron scattering and virus counting. *Structure* **11,** 1339 - 1348.

Lee, J. H., Wu, C. Y., Lee, C. N., Anwar, D., Wysocki, K. M., Lundgren, D. A., Farrah, S., Wander, J. and Heimbuch, B. (2009) Assessment of iodine-treated filter media for removal and inactivation of MS2 bacteriophage aerosols. *J Appl Microbiol*, In press.

Lee, J. H., Wu, C. Y., Wysocki, K. M., Farrah, S. and Wander, J. (2008) Efficacy of iodine-treated biocidal filter media against bacterial spore aerosols. *J Appl Microbiol* **105,** 1318-1326.

Lee, K.-K. and Ellis, A. E. (1991) A novel method for specific visualization of serum albumin in polyacrylamide gels by iodine staining. *Electrophoresis* **12,** 382–383.

Li, H. W., Wu, C. Y., Tepper, F., Lee, J. H. and Lee, C. N. (2008) Removal and retention of viral aerosols by a novel alumina nanofiber filter. *J Aerosol Sci* ***40,***65–71.

Lodish, H., Berk, A., Matsudaira, P., Kaiser, C. A., Krieger, M., Scott, M. P., Zipursky, S. L. and Darnell, J. (2003) Molecular Genetic Techniques and Genomics, New York, NY: W. H. Freeman and Company.

Mackay, I. M. (2004) Real-time PCR in the microbiology laboratory. *Clin Microbiol Infect* **10** (3)**,** 190-212.

Maillard, J. Y. (2001) Virus susceptibility to biocides: an understanding. *Rev Med Microbiol* **12** (2)**,** 63-74.

Malamud, D., Davis, C., Berthold, P., Roth, E. and Friedman, H. (1993) Human submandibular saliva aggregates HIV. *Aids Res Hum Retrov* **9** (7)**,** 633-637.

Marchin, G. L. and Fina, L. R. (1989) Contact and demand-release disinfectants. *CRC Crit Rev Environ Contr* **19** (4)**,** 277-290.

Marchin, G. L., Fina, L. R., Lambert, J. L. and Fina, G. T. (1983) Effect of resin disinfectants I_3 and I_5 on *Giardia muris* and *Giardia lamblia*. *Appl Environ Microbiol* **46** (5)**,** 965-969.

Marchin, G. L., Silverstein, J. and Brion, G. M. (1997) Effect of microgravity on *Escherichia coli* and MS-2 bacteriophage disinfection by iodinated resins. *Acta Astronautica* **40** (1)**,** 65-68.

Maus, R., Goppelsroder, A. and Umhauer, H. (1997) Viability of bacteria in unused air filter media. *Atmos Environ* **31** (15)**,** 2305-2310.

Maus, R., Goppelsroder, A. and Umhauer, H. (2001) Survival of bacterial and mold spores in air filter media. *Atmos Environ* **35** (1)**,** 105-113.

Mehrotra, R., Thornton, D. J. and Sheehan, J. K. (1998) Isolation and physical characterization of the MUC7 (MG2) mucin from saliva: evidence for self-association. *Biochem J* **334,** 415-422.

Messier, P. J. (2004) Method and system for treating air with an iodinated resin, US 6,696,055 B2, Triosyn Holdings, Inc.

Messier, P. J. (2009) manuscript in preparation.

MIPT (2002) The U.S. postal service response to the threat of bioterrorism through the mail. Oklahoma City, OK: National Memorial Institute for the Prevention of Terrorism.

Moir, A., Corfe, B. M. and Behravan, J. (2002) Spore germination. *Cell Mol Life Sci* **59,** 403-409.

Munro, L. (2000) Essentials for Microbiology Laboratory. Raleigh, NC: Contemporary Publishing Company of Raleigh, Inc.

Muus, J., Coons, A. H. and Salter, W. T. (1941) Thyroidal activity of iodinated serum albumin. IV. The effect of progressive iodination. *J Biol Chem* **139,** 135–144.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. and Setlow, P. (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* **64,** 548-572.

Nielsen, K. F., Thrane, U., Larsen, T. O., Nielsen, P. A. and Gravesen, S. (1998) Production of mycotoxins on artificially inoculated building materials. *Int Biodeter & Biodegrad* **42** (1)**,** 9-16.

NIOSH (2005) Determination of particulate filter penetration to test against liquid particulates for negative pressure, air-purifying respirators standard testing procedure (STP). Procedure No. RCT-APR-STP-0051, 0052, 0053, 0054, 0055, 0056, Revision 1.1. Pittsburgh, PA: National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory.

O'Connell, K. P., Bucher, J. R., Anderson, P. E., Cao, C. J., Khan, A. S., Gostomski, M. V. and Valdes, J. J. (2006) Real-time fluorogenic reverse transcription-PCR assays for detection of bacteriophage MS2. *Appl Environ Microbiol* **72** (1)**,** 478-483.

Oberdorster, G., Oberdorster, E. and Oberdorster, J. (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Persp* **113** (7)**,** 823-839.

OEHHA (2003) Technical support document: toxicology clandestine drug labs: Methamphetamine. Sacramento, CA: Office of Environmental Health and Hazard Assessment.

Opresko, L., Wiley, H. S. and Wallace, R. A. (1980) Proteins iodinated by the chloramine-T method appear to be degraded at an abnormally rapid rate after endocytosis. *Proc Natl Acad Sci* **77,** 1556–1560.

OSHA (1994) Iodine in workplace atmosphere (impregnated activated beaded carbon). Washington, DC: Occupational Safety & Health Administration.

OSHA (2000) Safety and health topics: Iodine. Washington, DC: Occupational Safety & Health Administration.

Pasanen, A. L., Keinanen, J., Kalliokoski, P., Martikainen, P. I. and Ruuskanen, J. (1993) Microbial-growth on respirator filters from improper storage. *Scand J Work Environ Health* **19** (6)**,** 421-425.

Pien, B. C., Saah, J. R., Miller, S. E. and Woods, C. W. (2006) Use of sentinel laboratories by clinicians to evaluate potential bioterrorism and emerging infections. *Clin Infect Dis* **42** (9)**,** 1311-1324.

Popham, D. L., Sengupta, S. and Setlow, P. (1995) Heat, hydrogen peroxide, and UV resistance of *Bacillus subtilis* spores with increased core water content and with or without major DNA-Binding proteins. *Appl Environ Microbiol* **61,** 3633-3638.

Portenier, I., Haapasalo, H., Rye, A., Waltimo, T., Ørstavik, D. and Haapasalo, M. (2001) Inactivation of root canal medicaments by dentine, hydroxyapatite and bovine serum albumin. *Intl Endodontic J* **34,** 184–188.

Prescott, L. M., Harley, J. P. and Klein, D. A. (2002) Microbiology, New York: McGraw-Hill.

Qian, Y., Willeke, K., Grinshpun, S. A. and Donnelly, J. (1997) Performance of N95 respirators: Reaerosolization of bacteria and solid particles. *Am Ind Hyg Asso J* **58** (12)**,** 876-880.

Qian, Y. G., Willeke, K., Grinshpun, S. A., Donnelly, J. and Coffey, C. C. (1998) Performance of N95 respirators: filtration efficiency for airborne microbial and inert particles. *Am Ind Hyg Assoc J* **59** (2)**,** 128-132.

Ratnesar-Shumate, S., Wu, C. Y., Wander, J., Lundgren, D., Farrah, S., Lee, J. H., Wanakule, P., Blackburn, M. and Lan, M. F. (2008) Evaluation of physical capture efficiency and disinfection capability of an iodinated biocidal filter medium. *Aerosol Air Qual Res.* **8,** 1-18.

Rengasamy, A., Zhuang, Z. P. and BerryAnn, M. S. (2004) Respiratory protection against bioaerosols: Literature review and research needs. *Am J Infect Control* **32** (6)**,** 345-354.

Ricca, E. and Cutting, S. M. (2003) Review emerging applications of bacterial spores in nanobiotechnology. *J Nanobiotechnol* **1,** 1-10.

Riemenschneider, L., Woo, M. H., Wu, C. Y., Lundgren, D., Wander, J., Lee, J. H., Li, H. W. (2009) Characterization of reaerosolization from impingers in an effort to improve airborne virus sampling, *J Appl Microbiol,* In press.

Schaffer, F. L., Soergel, M. E. and Straube, D. C. (1976) Survival of airborne influenza virus: effects of propagating host, relative humidity, and composition of spray fluids. *Arch Virol* **51** (4)**,** 263-273.

Seinfeld, J. H. and Pandis, S. N. (1998) Atmospheric Chemistry and Physics, New York, NY: John Wiley & Sons, Inc.

Shahkrokh, B. K. (1943) Preparation and antigenic properties of a crystalline labeled antigen. *J Biol Chem* **151,** 659–664.

Simmons, R. B. and Crow, S. A. (1995) Fungal colonization of air filters for use in Heating, Ventilating, and Air-Conditioning (HVAC) Systems. *J Indus Microbiol* **14** (1)**,** 41-45.

Small, B. M. (2002) Indoor air pollutants in residential settings: respiratory health effects and remedial measures to minimize exposure. *A Review of the Ontario Lung Association*.

Smith, M. F., Bryant, S., S., W. and Digenis, G. A. (1984) Labeling suspended aerosol particles with short-lived radionuclides for determination of particle deposition. *J Pharm Sci* **73** (8)**,** 1091–1094.

Sobsey, M. D., Oldham, C. E. and McCall, D. E. (1990) Comparative inactivation of hepatitis a virus and other enteroviruses in water by iodine. *Water Sci Technol* **24** (2)**,** 331-337.

Songer, J. R. (1967) Influence of relative humidity on survival of some airborne viruses. *Appl Microbiol* **15** (1)**,** 35-42.

Stokley, P. G., Stonehouse, N. J. and Valegard, K. (1994) Molecular mechanism of RNA phage morphogenesis. *Int J Biochem* **26** 1249-1260.

Taylor, S. L., Fina, L. R. and Lambert, J. L. (1970) New water disinfectant - an insoluble quaternary ammonium resin-triiodide combination that releases bactericide on demand. *Appl Microbiol* **20** (5)**,** 720-722.

Tennen, R., Setlow, B., Davis, K. L., Loshon, C. A. and Setlow, P. (2000) Mechanisms of killing of spores of *Bacillus subtilis* by iodine, glutaraldehyde and nitrous acid. *J Appl Microbiol* **89,** 330-338.

Thermo Electron Corporation (2003) Six and two stage viable samplers: Instruction manual. Asheville, NC: Thermo Electron Corporation.

Trouwborst, T. and de Jong, J. C. (1973) Interaction of some factors in mechanism of inactivation of bacteriophage-MS2 in aerosols. *Appl Microbiol* **26** (3)**,** 252-257.

Tyrrell, D. A. J. (1967) The spread of viruses of the respiratory tract by the airborne route. *Symp Soc Gen Microbiol* **17,** 286-306.

U.S. Army (1998) Filter medium, fire-resistant, high efficiency, Military Specification MIL-F-51079D, Aberdeen Proving Ground, MD: U.S. Army Armaments Munitions and Chemical Commands.

van der Strate, B. W. A., Beljaars, L., Molema, G., Harmsen, M. C. and Meijer, D. K. F. (2001) Antiviral activities of lactoferrin. *Antivir Res* **52** (3)**,** 225-239.

Veerman, E. C. I., van den Keybus, P. A. M., Vissink, A. and Nieuw Amerongen, A. V. (1996) Human glandular salivas: their separate collection and analysis. *Eur J Oral Sci* **104,** 346-352.

Vissink, A., Waterman, H. A., Sgravenmade, E. J., Panders, A. K. and Vermey, A. (1984) Rheological properties of saliva substitutes containing mucin, carboxymethylcellulose or polyethylenoxide. *J Oral Pathol Med* **13** (1)**,** 22-28.

Walker, D. C., S.-V. Len and Sheehan., B. (2004) Development and evaluation of a reflective solar disinfection pouch for treatment of drinking water. *Appl Environ Microbiol* **70,** 2545-2550.

Wang, Z., Reponen, T., Grinshpun, S. A., Gorny, R. L. and Willeke, K. (2001) Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. *J Aerosol Sci* **32,** 661-674.

Wang, Z., Reponen, T., Willeke, K. and Grinshpun, S. A. (1999) Survival of bacteria on respirator filters. *Aerosol Sci Technol* **30** (3)**,** 300-308.

Willeke, K. and Qian, Y. G. (1998) Tuberculosis control through respirator wear: performance of national institute for occupational safety and health-regulated respirators. *Am J Infect Control* **26** (2)**,** 139-142.

Willeke, K., Qian, Y. G., Donnelly, J., Grinshpun, S. A. and Ulevicius, V. (1996) Penetration of airborne microorganisms through a surgical mask and a dust/mist respirator. *Am Ind Hyg Assoc J* **57** (4)**,** 348-355.

Wong, L. and Sissions, C. H. (2001) A comparison of human dental plaque microcosm biofilms grown in an undefined medium and a chemically defined artificial saliva. *Arch Oral Biol* **46,** 477-486.

Wyss, O. and Strandskov, F. B. (1945) The germicidal action of iodine. *Arch Biochem* **6** (2)**,** 261-268.

Zalewska, A., Zwierz, K., Zolkowski, K. and Gindzienski, A. (2000) Structure and biosynthesis of human salivary mucins. *Acta Biochim Pol* **47** (4)**,** 1067-1079.

BIOGRAPHICAL SKETCH

Jin-Hwa Lee was born in South Korea, to Jun-Gil Lee and Young-Soon Kim in 1977. She received a B.S degree from the Department of Environmental Science and Engineering at the Keimyung University in 2000. After graduation, she worked at the Korea Environmental Research Incorporation as an engineer from 2001 to 2004. While working for the company, she enrolled in the master's program of the Environmental Health at the Yonsei University and received the degree in 2003.

She entered the Ph.D program of the Environmental Engineering and Sciences at the University of Florida from fall 2004. She worked with Dr. Chang-Yu Wu, as a research assistant in the Aerosol and Particulate Research Laboratory (APRL). Her current study involves characterization of virus aerosols generated from various spray media. She received her Ph.D. from the University of Florida in the summer of 2009.